
Effects of Ground-Level Ozone on Vegetation in the UK
Defra Contract 1/3/201
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Ozone Umbrella:

Effects of Ground-level Ozone on (Upland) Vegetation in the UK

1/3/201

Final Report 2006

CEH C02158 Report No. AS 06/02

Executive Summary

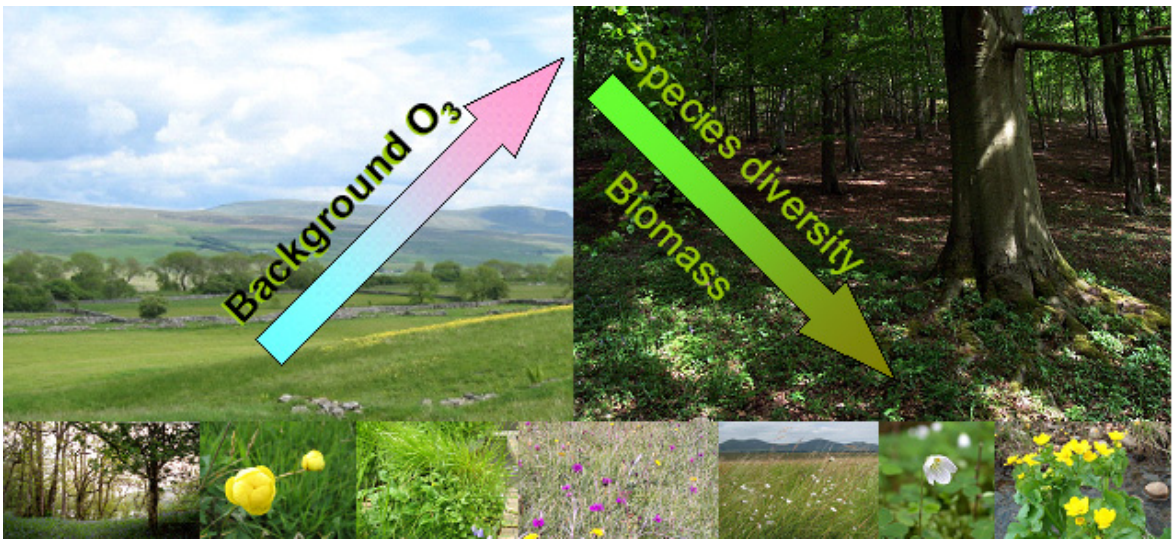
Introduction

This report describes the work done under contract 1/3/201 to examine the effects of ground-level ozone on individual plants and communities in the UK. There is a particular focus on upland vegetation as previous studies (Coyle *et al* 2003; Ashmore *et al* 2002) had shown that they may be at particular risk of damage as background ozone concentration increase. A substantial amount of data on ozone effects and fluxes to vegetation has been compiled and published in the literature or presented at scientific meetings (see Outputs). The project was split into 5 work packages the results of which are described in the individual chapters and summarised below.

Overall it was found that many species of conservation value are sensitive to ozone at relatively low concentrations and drought may exacerbate effects in some:

- Conservative increases in ozone concentration (typical of predictions for background ozone levels; from 30 ppb to 50 ppb during the daytime by 2050) resulted in significant shifts in species composition of a species rich upland meadow with the rare grasses being suppressed in favour of a common opportunistic grass.
- Exposure to ozone at night or at cold temperatures has a disproportionately adverse effect on plant growth, increasing the risk of damage to upland species.
- Plant communities of upland woodlands, including characteristic species, may be relatively sensitive to ozone.
- Some species showed carry-over effects of ozone exposure from one summer to the following spring, highlighting the importance of long-term effects studies.

There follows a more detailed summary of the results from all components of the work package.



Effects of Ozone on Upland, Woodland and Mire Species

- Conservative increases in ozone concentrations, as predicted for the UK over the next 30-50 years, resulted in significant shifts in the composition of a species-rich upland meadow community within a relatively short period (12-14 months to yield statistically discernible changes); with rare grasses of high conservation value (*Phleum* and *Briza*) suppressed in favour of an opportunistic grass (*Alopercurus*)
- Evidence of substantial night-time conductance in a significant fraction of the upland flora was found.
- Exposure to ozone at night or at low temperature was shown to have a disproportionately adverse effect on plant growth, this may indicate a need to 'weight' ozone exposures (e.g. day/night and by season), when considering risk assessment approaches for upland vegetation
- Species or populations collected from Scar Close in the Yorkshire Dales showed no negative responses to ozone fumigation. This suggests that limestone pavement habitats may be less responsive to ozone. Possibly the extreme nutrient and water stress in this habitat lead to adaptations that also increase resistance to ozone.
- There were few significant effects on above ground biomass, and these were both positive and negative. However, a range of other growth and morphological responses to ozone were observed. Hence, reliance on measurements of above-ground biomass to assess ecological responses to ozone may be misleading.
- Root biomass and root:shoot ratio were consistently lower in ozone, with five species showing significant negative effects. In communities on soils with low nutrient status and low water-holding capacity, as is common in the Yorkshire Dales National Park, such effects may have adverse ecological consequences.
- Several species showed significant changes in leaf morphology in response to ozone treatment. The significance of these changes in an ecological context will depend on the particular niches and habitats occupied by the species.
- Individual species and simulated communities of upland plants from Snowdonia, North Wales were exposed to episodic peak and increased background ozone concentrations. Several individual species showed visible injury, premature senescence and/or changes in above ground biomass when exposed to episodic peaks. Simulated communities that were exposed to increased background ozone concentrations showed early senescence and significant reductions in biomass.
- Some species that showed no effects during the ozone exposure did have carry-over effects on biomass the following spring, after a winter period in ambient ozone concentrations. Such carry-over effects indicate the potential ecological impact of ozone on semi-natural species and the importance of long-term effects studies.

- Plant communities of upland woodland habitats may be relatively sensitive to ozone. The adverse effects of ozone were particularly marked on the more characteristic woodland species, but the overall effect on community structure may depend on the micro-habitat within the woodland. At open clearing or edge sites, the outcome of competition may be modified by ozone to favour invasive species. However, in denser shade, with less competition, it is the direct effect of ozone on the long-term viability of populations of woodland species that may be of greater significance.
- Although there is some evidence of effects of ozone on mire communities after less than one year of exposure, we consider that continuation of the experiment for a further 2-3 years is essential to evaluate longer-term effects on this mire community. Detailed studies on the underlying mechanisms that cause these ozone responses will help to improve our understanding of how increases in ozone concentrations may influence plant communities and the carbon budget of mire ecosystems in the future.

Interactive Effects of Ozone Exposure and Drought

- This study focused on physiological changes (in stomatal conductance and photosynthetic rate), which may occur independently of any significant reductions in growth rate but will potentially impact upon them. There was no evidence of oxidative damage causing any reductions in the photosynthetic capacity of the four species studied. Nevertheless, the more subtle physiological changes via modification of stomatal functioning and plant water relations may play an important role in patterns of interspecific competition
- The legume species Lotus appeared to be essentially unaffected by exposure to elevated ozone concentration, both in general and in terms of its physiological response to soil drought. In contrast, *Trifolium* consistently reduced its stomatal conductance above a certain cumulative level of exposure to ozone, both when well-watered and during periods of drought, which would also result in reduced uptake of ozone by the leaves, and increased water conservation during periods of drought. Thus, the risk of both oxidative and water stress may be substantially reduced over the course of a growing season.
- The response of *Phleum* to combined ozone and drought stress was potentially very significant. Exposure to elevated ozone consistently reduced the strength of its stomatal response to soil drying. During long periods of drought, when exposed to high ozone levels, *Phleum* is likely to experience damaging levels of water deficit. At the same time, the lack of stomatal closure will simultaneously increase ozone uptake - the combination of which could be very damaging and would be expected to impact on both structure and functioning of the plant community. In the context

of changing rainfall patterns under climate change, it is likely that responses of this kind will have important effects on vegetation structure and functioning. We have just begun to assess the interacting influence of ozone exposure and soil drying on the up-regulation of anti-oxidant systems. This topic will receive further attention in the up-coming project.

Field Measurements of Ozone Flux

- A substantial dataset of ozone flux measurements for an upland moor/bog (Auchencorth Moss) and improved grassland (Easter Bush) has been compiled, as well as shorter periods of measurements over an oak forest (Alice Holt) and semi-natural upland grassland (Colt Park). The data include estimates of canopy conductance and so stomatal ozone uptake.
- The sites with the greatest total ozone deposition are Alice Holt and Easter Bush. At the latter, this is due to the grassland (mainly *Lolium perenne*) being highly productive with an associated large stomatal conductance during the growing season. This would increase its ability to out-compete other species at higher ozone concentrations given it is not very sensitive to ozone compared to some other grasses, as was noted in Section 4.
- At the upland grassland site, much larger values of of stomatal conductance than anticipated were measured. It is likely that there was some interference from non-transpired water-vapour which would have increased the estimates of stomatal flux. This showed the importance of measuring for long periods to obtain data in suitable conditions as well as the accurate determination of canopy wetness.
- Stomatal uptake varied between 5 to 60% of the total depending on season and the vegetation. For a whole year upland moor/bog consistently had the smallest stomatal uptake (5-20%), followed by the grassland (20-40%) although during the summer months their could be periods with values over 80%.
- Non-stomatal ozone deposition was found to vary with surface temperature, solar radiation, friction velocity and relative humidity depending on surface conditions.
- For dry surfaces, deposition increases with increasing surface temperature and solar radiation which supports the hypothesis that ozone is destroyed on leaf surfaces by thermal decomposition, mediated by compounds on or emitted by the leaf surface and that some photochemical/photolytic reactions also occur.
- For wet surfaces deposition was greater than over dry indicating the water films on vegetation are an efficient sink for ozone. The results indicate that there are aqueous chemical reactions occurring and that additional photochemical reactions may occur in daylight. A simple model of $O_3/SO_2/NH_3$ showed that if sufficient NH_3 was present to increase the pH of a water film above 7 then SO_2/O_3 oxidation could be a significant sink for ozone.

Outputs

Hayes, F., Mills, G., Williams, P., Harmens, H., Büker, P. Impacts of summer ozone exposure on the growth and overwintering of UK upland vegetation. Atmospheric Environment, in press.

Cape J.N., Coyle M., Fowler D. (2004): Ozone in a changing world – what do we need to know? 36th US Air Pollution Workshop, Rhinelaner, Wisconsin

Mhairi Coyle, Mike Ashmore, David Fowler, Felicity Hayes and Ron Smith. Trends in Ground-level Ozone Concentration and Their Implications for the UK. 13th WORLD CLEAN AIR AND ENVIRONMENTAL PROTECTION CONGRESS AND EXHIBITION London, UK, August 22-27, 2004

Trends In Ground-level Ozone Concentration and their Implications For Scottish Vegetation. Terrestrial Environmental Change in the Highlands and Islands: From Mountain Summits to Coasts Inverness, 2004. Mhairi Coyle, Mike Ashmore, David Fowler, Felicity Hayes and Ron Smith

What Controls Non-Stomatal Ozone Depoistion?. Acid Rain 2005, Prague, Czech Republic, 12th – 17th June 2005

Mills, G., Hayes, F., Williams, P, Jones, M.L.M., Macmillan, R., Harmens, H., Lloyd, A., Büker, P. 2005. Should the effects of increasing background ozone concentration on semi-natural vegetation communities be taken into account in revising the critical level? Background paper workshop on 'Critical levels of ozone: further applying and developing the flux-based concept', 15-19 November 2005, Obergurgl, Austria.

Significant UK dataset contributing to the refinement of critical exposures for the protection of long-lived plant communities from ozone pollution (via UNECE Workshop at Obergugl, Austria [2005] and ICP-Vegetation Workshop Bangor [2006])

Bassin S, Volk M, Fuhrer J (2006) Predicting the sensitivity to ozone of temperate European grasslands: an overview. *Environmental Pollution*, In Press

Contents

1 Impacts of Ozone on Species in Different Upland Communities

University of York: Mike Ashmore and Becky Keelan

1.1 Introduction

The aim of this work package was to develop an increased understanding of the impacts of ozone on species of a range of different upland communities. The majority of the work was focussed on controlled laboratory experiments, supported by field observations, related to one important area of high conservation value – the Yorkshire Dales National Park. In this region, as in most such areas in the UK, a range of different plant communities are found, reflecting variation in soils, microclimate and management history. Some modifications of the original work programme have been made, reflecting partly difficulties with planned work, and partly the interest in investigating particular aspects of the findings which were judged to be of high value.

1.1.1 Introduction to study region and sites

Sites were chosen to represent habitats typical of the upland areas of the Yorkshire Dales National Park, including grasslands, woodlands and limestone pavement. It was also important to include sites that were botanically diverse and representative of endangered and protected UK habitats. All sites that were selected had SSSI status. Six initial sites were selected for study, as follows:-

Yockenthwaite Meadows are located on steep south facing slopes from adjacent to the river to an altitude of about 300m. Yockenthwaite Meadows are managed as traditional hay meadow, and are particularly species rich and diverse with typical calcicolous grassland species. The NVC classification is MG3 and MG5, upland hay meadow.

Conistone Old Pastures covers a large area on the eastern flanks of the Wharfe valley. They are diverse in habitat type ranging from managed pasture, at the lower reaches, to grassland and limestone pavement in the upper reaches with scattered areas of moorland. The area includes several NVC communities, including CG9, CG2, MG5 and MG6.

Ingleborough Limestone Complex - Scar Close is an area of limestone pavement within the Ingleborough limestone complex. It features a diverse habitat ranging from typical limestone pavement to islands of acidic peat. The depth of the grikes varies considerably across the site; there are some very deep grikes unable to support any plant life, and shallower grikes, which provide suitable habitat for shade-tolerant woodland species.

Ingleborough Limestone Complex – Ribblehead (Colt Park) is a barn situated adjacent to Colt Park SSSI. The fields surrounding the office belonging to English Nature are limestone pavement and meadow, and are grazed and cut for hay. The site is not particularly rich in species but does provide some of the properties of the nearby SSSI, and was selected because it provided facilities for ozone monitoring.

Grass Wood is mixed oak-ash woodland on limestone. The gradient is steep and it runs down to the river. It is notably rich in species, with many common woodland species. Its particular geography allows higher species diversity due to the habitat mosaic. There is a small area of the woodland which is being traditionally coppiced.

Bastow Wood is very different in geography and physiognomy to Grass Wood. The woodland is more like wood pasture with much open space. The sward is generally low to the ground and is mainly controlled by grazing rabbits; sheep also graze at the site from time to time. The habitat diversity is good, ranging from bare limestone to wood-pasture.

1.2 Assessment of ozone response of species typical of different upland plant communities

1.2.1 Introduction and aims

Spatial differences in response to ozone within an upland region of conservation importance depend on a number of factors. One of the most important of these is the sensitivity of the individual species found in each community. Furthermore, although there is evidence in the literature of differences in ozone sensitivity between populations of the same species collected from different locations, none of these studies have specifically tested whether this is related to the habitat. Although most studies of plant responses to ozone have focussed on effects above ground, effects on root growth may be of considerable ecological significance in the characteristically nutrient limited communities of upland Britain.

Therefore, the aims of this part of the investigation were to:- (a) identify sensitive species of upland communities of this area; (b) compare ozone sensitivity of species and populations of different habitats; and (c) to assess if the effects of ozone were greater on below-ground than above-ground growth. Plants were grown in the laboratory from seed that was collected at the six sites, and hence the reported comparisons of species and habitats do not reflect any differences between habitats during growth and ozone exposure.

1.2.2 Methods

1.2.2.1 Fumigation system and exposure conditions

The ozone fumigation system comprised eight, 90 x 90 x 90 cm Perspex chambers each with its own regulated air supply from a dedicated air conditioning system. Temperature

and humidity were set a constant level which was different for day and night and regulated by a sensor and feedback system. Four chambers were fed with charcoal filtered air and 4 with a target concentration of 80ppb of ozone. Ozone levels were manually set and monitored with a calibrated ozone monitor.

1.2.2.2 Collection of seed and plant growth

Seeds were collected from wild populations from the above sites, laid out to dry, sorted and stored in envelopes until required. Seeds were sown into Petri dishes, on dampened filter papers and placed under light to germinate. Prior to light treatment some seeds were given 6 weeks in the freezer at 40C to break dormancy. The final selection of species used in this study was ultimately determined by the success of germination and propagation. After germination, seedlings were transferred to seed trays containing John Innes seed compost and grown for 2-3 weeks in growth chambers. The plants were then transferred on to pots containing a potting mixture of 70% John Innes No2 loam-based compost and 30% limestone chippings to simulate the calcareous soils present in this upland region.

1.2.3 Results

A total of 14 native species and populations of the Yorkshire Dales were assessed; three species, namely Lotus corniculatus, Plantago lanceolata and Sanguisorba minor, were present at more than one site and thus it was possible for more than one population to be fumigated. Rather than simply focussing on above-ground biomass, a range of growth indices were measured at the end of each fumigation period; the parameters varied according to the particular growth characteristics of each species. Table 2.1 summarises the direction and statistical significance of the observed responses; the text below summarises some of the key findings.

1.2.3.1 Visible injury and leaf senescence

Characteristic symptoms of ozone injury (stipple and flecking) were observed only on three species (*Geranium lucidum*, *Glechoma hederacea* and *Scrophularia nodosa*). A significant increase in dead leaf biomass was observed for all three populations of *Sanguisorba minor* and for *Scrophularia nodosa*.

1.2.3.2 Above ground biomass

Percentage differences between the control and ozone treatments for above-ground biomass are shown in Figure 1.1. Only the *Lotus corniculatus* population from Conistone Old Pastures showed a significant (32%) decrease in above ground biomass. Two other *L.corniculatus* populations showed smaller, non-significant decreases in above ground biomass, whereas the Scar Close population displayed a non-significant 6% increase in ozone. All three of the *Sanguisorba minor* populations showed a positive response under

ozone conditions; the population from Bastow Wood showed the greatest increase (23%) in above-ground biomass.

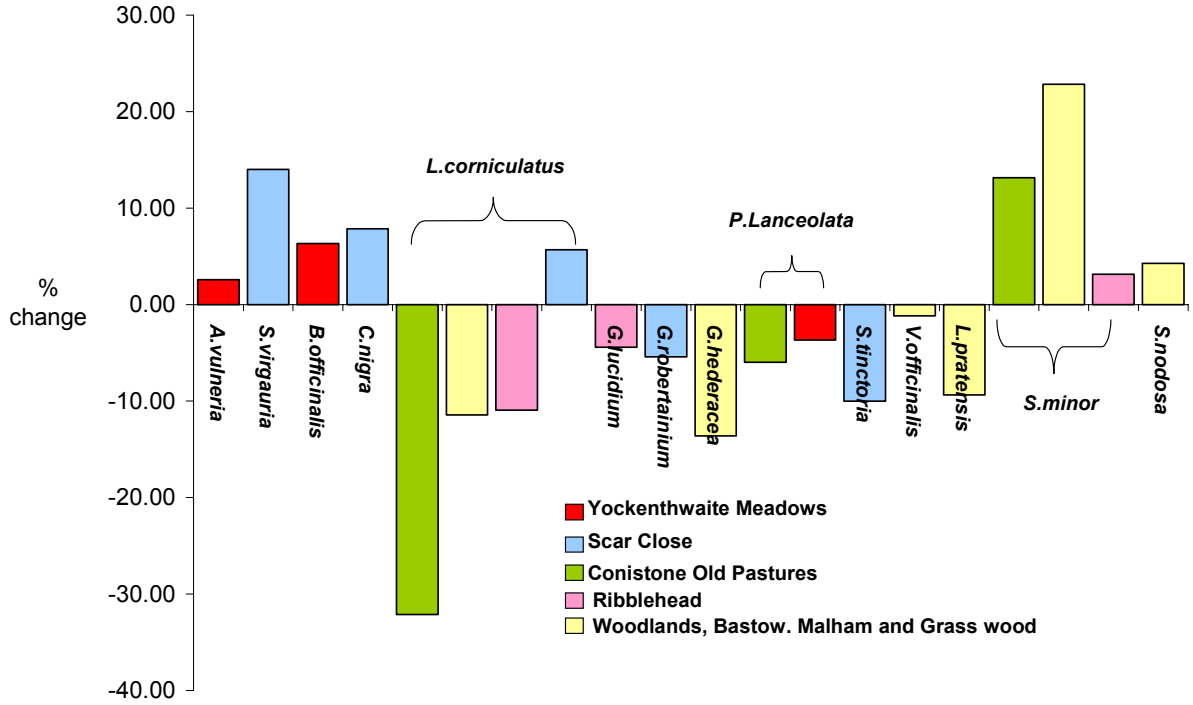


Figure 1.1 The percentage difference in mean above-ground biomass between ozone and filtered air treatments (a negative value indicates a reduction in ozone).

1.2.3.3 Root biomass

Root biomass is the parameter for which the greatest adverse effects of ozone were found (Figure 1.2). Three species showed a significant decrease in root biomass, these were *G.lucidium, G.hederacea*, and two populations of *L. corniculatus* (Conistone Old Pastures and Bastow Wood). *Anthyllis vulneria* showed the largest decrease in mean root biomass, of 56%. No species showed a significant positive effect of ozone on root biomass.

1.2.3.4 Root:shoot ratio

Responses of the root:shoot ratio relate directly to the changes in root biomass, as there was relatively little change in above ground biomass. Three species showed a significant decrease in the root to shoot ratio; these were *A.vulneria*, *G.lucidium* and *L.corniculatus*.

1.2.3.5 Leaf number, area and morphology

In general, differences in the mean number of leaves per plant between treatments were small. One population of *L.corniculatus* (Ribblehead) showed a significant 16% decrease in mean leaf number, but the other three populations had no significant response. *S.minor* also responded to ozone treatment but with a positive response. All three populations showed an increase in number of leaves; the Ribblehead population showed

the largest and significant increase of 20%, while the Conistone Old Pastures and Bastow Wood populations showed non-significant increases of 15% and 14% respectively.

G.hederacea showed the most interesting response to ozone conditions in terms of leaf morphology and growth parameters (Figure 1.3). The mean leaf length was significantly increased by 20% in ozone, while the mean individual leaf area also significantly increased in ozone, by 29%. In contrast, the total leaf area showed no effect of treatment, with a difference of only 0.03%, while the mean number of leaves per plant decreased significantly, by 33%, in ozone. Thus, overall the ozone fumigated plants have fewer but larger leaves.

Leaf width was only measured in the two populations of *Plantago lanceolata*, due to the known phenotypic plasticity of this species. There was a significant decrease in the width of the leaves in ozone in both populations, by 14% in the Conistone Old Pastures population and by 10% in the Yockenthwaite Meadows population.

Other than *G.hederacea*, only *G.lucidium* showed a significant response in individual leaf area. Ozone decreased the mean individual leaf area by 13% and also decreased total leaf area by 14%. The Scar Close population of *L.corniculatus* showed a significant 16% increase in total leaf area, in contrast to the other three populations which all showed non-significant decreases in leaf area. All three *S.minor* populations showed an increase in total leaf area in ozone, despite ozone causing an increase in leaf senescence. This effect was significant for the Conistone Old Pastures population, which showed a 47% increase in ozone.

1.2.4 Conclusions

- Six of the fourteen species showed no significant response of any parameter to ozone treatment.
- None of the species or populations collected from Scar Close showed any negative response to ozone fumigation. This suggests that limestone pavement habitats may be less responsive to ozone. Possibly the extreme nutrient and water stress in this habitat lead to adaptations that also increase resistance to ozone.
- The extent of variation between populations varied with species. Three populations of *Lotus corniculatus* showed a negative response to ozone treatment, but the Scar Close population showed only one response and this was a positive one, increased leaf area. In contrast, both populations of *Plantago lanceolata* showed morphological sensitivity to ozone and all three populations of *Sanguisorba minor* showed similar types of responses to treatment with ozone.
- There were few significant effects on above ground biomass, and these were both positive and negative. However, a range of other growth and morphological

responses to ozone were observed. Hence, reliance on measurements of above-ground biomass to assess ecological responses to ozone may be misleading.

- Two species showed significant increases in senescence in ozone, but these were not associated with adverse effects of ozone on growth; indeed for *Sanguisorba minor*, they were associated with increased above-ground biomass.
- Root biomass and root:shoot ratio were consistently lower in ozone, with five species showing significant negative effects. In communities on soils with low nutrient status and low water-holding capacity, as is common in the Yorkshire Dales National Park, such effects may have adverse ecological consequences.
- Several species showed significant changes in leaf morphology in response to ozone treatment. The significance of these changes in an ecological context will depend on the particular niches and habitats occupied by the species.

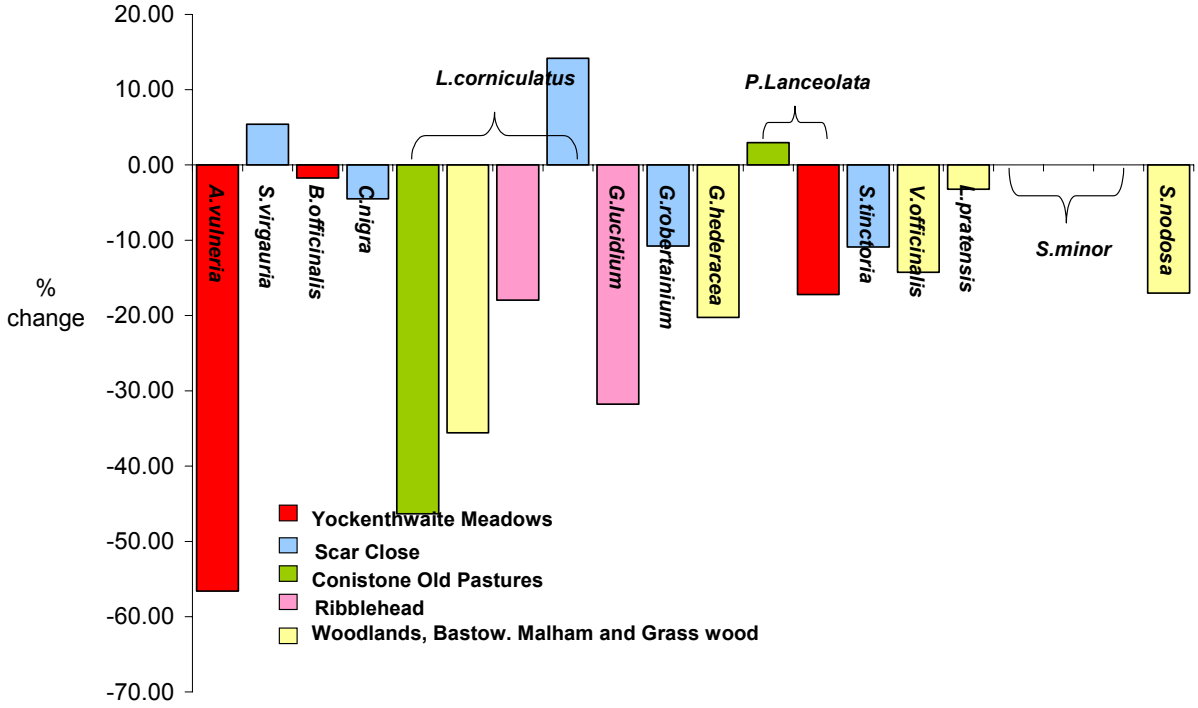


Figure 1.2 The percentage difference in mean root biomass between ozone and filtered air treatments (a negative value indicates a reduction in ozone).

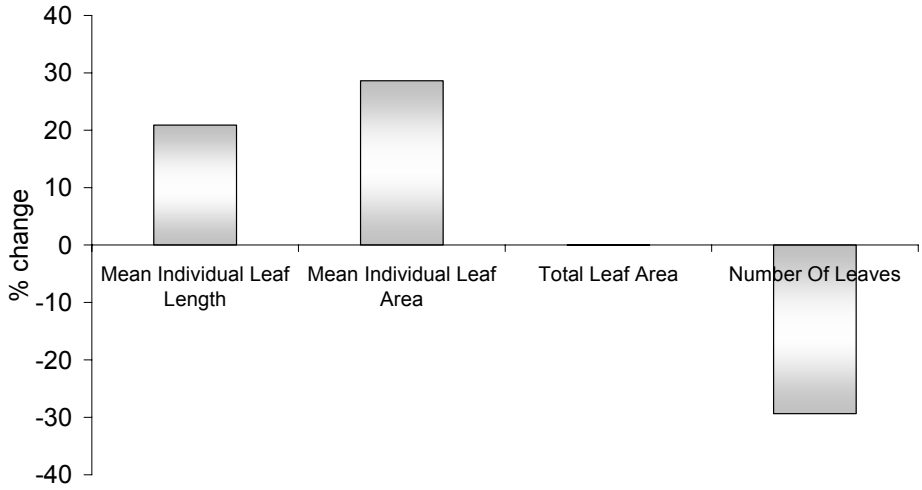


Figure 1.3 The effect of ozone fumigation on leaf morphology (shown as percentage difference between ozone and control) for Glechoma hederacea

*Table 1.1. Summary of results for assessment of response to ozone. – indicates that the parameter was not measured, NS indicates that the effect of ozone was not significant at P=0.05, and *, ** and *** indicate significance at P<0.10, P<0.05 and P<0.01 respectively.*

Species	Site Of Collection	Measured Indices																
		Biomass							Leaf Number	Leaf Area		Leaf Length	Leaf Width	Stem Length	Number of Stems	Stomatal conductance	Dead Leaves	Visible Injury
		Upper				Roots	Total	R:S		Individual Leaf	Total							
		Leaves	Buds	Stems	Flowers													
Anthyllis vulneria	Yockenthwaite meadows	NS	-	-	-	↓*	NS	↓**	NS	-	NS	NS	-	-	-	-	-	NONE
Betonica officinalis	Grass Wood	NS	-	-	-	NS	NS	NS	NS	NS	NS	-	-	-	-	-	-	NONE
Centurea nigra	Scar Close	NS	-	-	-	NS	NS	NS	NS	NS	NS	-	-	-	-	-	NS	NS
Geranium lucidium	Ribblehead	NS	-	-	-	↓***	NS	↓***	NS	↓***	↓**	-	-	-	-	↑***	NS	↑***
Geranium robertainium	Scar Close	NS	-	-	-	NS	NS	NS	NS	NS	NS	-	-	-	-	-	NS	NONE
Glechoma hederacea	Malham	NS	-	NS	-	↓**	NS	NS	↓**	↓***	NS	↓***	-	NS	NS	NS	NS	↑***
Lathyrus pratensis	Ribblehead	NS	-	NS	-	NS	NS	NS	NS	-	-	-	-	NS	NS	-	NS	NONE
Lotus corniculatus	Conistone Old pastures	↓**	-	-	-	↓**	↓***	NS	NS	-	NS	-	-	NS	NS	-	-	NONE
Lotus corniculatus	Bastow Wood	NS	-	-	-	↓***	NS	↓***	NS	-	NS	-	-	NS	NS	-	-	NONE
Lotus corniculatus	Ribblehead	NS	-	-	-	NS	NS	NS	↓*	-	NS	-	-	NS	NS	-	-	NONE
Lotus corniculatus	Scar Close	NS	-	-	-	NS	NS	NS	NS	-	↑*	-	-	NS	NS	-	-	NONE
Plantago lanceolata	Conistone Old pastures	NS	-	-	-	NS	NS	NS	NS	NS	NS	NS	↓**	-	-	-	NS	NONE
Plantago lanceolata	Yockenthwaite meadows	NS	-	-	-	NS	NS	NS	NS	NS	NS	NS	↓**	-	-	-	NS	NONE
Sanguisorba minor	Conistone Old pastures	NS	-	-	-	-	-	-	NS	-	↑**	-	-	-	-	-	↑**	NONE
Sanguisorba minor	Bastow Wood	↑*	-	-	-	-	-	-	NS	-	NS	-	-	-	-	-	↑**	NONE
Sanguisorba minor	Ribblehead	NS	-	-	-	-	-	-	↑**	-	NS	-	-	-	-	-	↑**	NONE
Scrophularia nodosa	Grass Wood	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	-	-	-		↑***	↑***
Serratula tinctoria	Scar Close	NS	-	-	-	NS	NS	NS	NS	NS	NS	NS	-	-	-	NS	-	NONE
Solidago virgireau	Scar Close	NS	-	-	-	NS	NS	NS	NS	-	NS	NS	-	-	-	-	-	NONE
Valeriania officinalis	Grass Wood	NS	-	-	-	↓*	NS	↓**	NS	NS	NS	NS	-	-	-	-	-	NONE

1.3 Responses of woodland communities

1.3.1 Introduction and aims

An important finding from the experiments described above is the sensitivity to ozone of a number of species of conservation importance in woodlands. Much previous research has focussed on species of grasslands and other open habitats, but little is known of the effects of ozone on woodland ground flora. Nevertheless, these woodland communities are of high conservation value, and the spring flowers of upland deciduous woodlands have major public appeal. For these reasons, we modified the original programme to conduct more detailed experimental studies of ozone impacts on these important communities.

In designing these experiments, it was important to consider several important aspects of woodland ecology. Firstly, there are a number of different micro-habitats within a wood, and indeed management for conservation often aims to create a range of habitats to increase species diversity. Secondly, the degree of shade, and the ability of species to adapt to low irradiance is the major determinant of community composition. Thirdly, most woodland ground flora species develop from seed or rhizomes each year, and the rate of emergence and growth in spring may be crucial.

Therefore, the major aims of the three experiments were:- (a) to determine if ozone significantly affected the species composition of woodland ground flora; (b) to assess if the degree of shade modified the impacts of ozone; (c) to determine if there were systematic shifts between shade tolerant and invasive species, using the Ellenberg classification system; and (d) to identify woodland species that are particularly sensitive to ozone.

1.3.2 Summary of methods

Soil was collected from various sites within Grass Wood and used to establish mesocosms using the seed and rhizome source within it. This was done to ensure that a community structure was produced that was similar to the woodland from which the soil had been taken. To simulate the growing woodland canopy, the mesocosms were shaded after 4 weeks, and then this was increased after a further 4 week period. In the initial experiment all mesocoms were shaded. Experiments 2-3 also had control mesocosms with no shade, to test the hypothesis that ozone effects would be modified by shade. The mesocosms were randomly distributed between the eight experiment chambers; four of these received a concentration of 80ppb ozone (8h day_{-1}) and four received filtered air.

Table 1.2 summarises the main features of the experiment. Experiments 1 and 3 used a relatively open wet area of the site, while Experiment 2 was taken from a dense regenerating coppice area; much fewer species emerged from this coppice soil. The

collection dates differed, which could create differences in the quantity of available and viable seed. In general, the emergence and growth rates in Experiment 1 were greater than in Experiments 2 and 3, leading to greater degrees of competition within the mesocosms.

Table 1.2. Summary of the three woodland mesocosm experiments

	Collection Site	Date of Collection	Fumigation Length (days)	No. Mesocosms	Treatments	Species Richness
1	Site 1 – Open wet area of woodland	Oct 04	98	17	Ozone/Control	40
2	Site 2- Closed 7-8 year old Coppice plot	Mar 05	149	41	Light – Ozone/Control Shade – Ozone/Control	14
3	Site 1	Apr 05	105	39	Light – Ozone/Control Shade – Ozone/Control	30

1.3.3 Results

Table 1.3 provides a summary of the overall effects of ozone on major community level response variables. Visible foliar injury was consistently found in all three experiments, but otherwise the results showed major differences between the three experiments. In Experiment 2, ozone caused a significant overall effect on cover, and also a decrease in the number of species present, the Shannon-Weaver diversity index (H') and an equitability index Eh. In contrast, ozone caused an increase in species richness and abundance in Experiment 1, and no significant effects in Experiment 3.

*Table 1.3 Summary of analysis of variance of three woodland experiments. Arrows indicate a significant of ozone, or a significant ozone*time interaction, at P=0.05, with an upward arrow indicating an increase in ozone and a downward arrow indicating a decrease in ozone. ns indicates no significant effect, ~ indicates no consistent trend in ozone effects over time*

	Woodland experiment					
	1		2		3	
	O_3	O_3 *Time	O_3	O_3 *Time	O_3	O_3 *Time
Species Richness	↑	ns	↓	↓	ns	ns
Abundance	↑	ns	↓	ns	ns	ns
H'	ns	~	ns	↓	ns	ns
Eh	ns	~	ns	↓	ns	ns
Foliar Injury	↑	↑	↑	↑	↑	↑
Percentage Cover	ns	ns	↓	↓	ns	ns

Table 1.4 summarises the species showing visible injury. Many species showed accelerated senescence and chlorosis, or developed red colouration, but these sympotoms were found in both treatments are not ozone-specific. Three species (*Glechoma hederacea, Hypericum humifusum* and *Rubus spp*.) showed typical flecking and stipple in all three experiments, while *Schrophularia nodosa* and *Veronica*

chameadrys showed such injury in the one experiment in which they were found. The other listed species showed injury in one experiment but not in others.

Table 1.4. Summary of species showing visible injury. X indicates species showing typical symptoms of stipple or fleck, 0 indicates species present but no ozone specific injury – indicates species not present

Species	Expt. 1	Expt. 2	Expt. 3
Glechoma hederacea	x	x	x
Holcus lanatus	x	-	0
Hypericum humifusum	x	x	x
Lysimachia nummularia	x	-	0
Plantago lanceolata	x	-	0
Potentilla sterilis	0	x	x
Rubus spp	x	x	x
Ranunculus repens	0	-	x
Scrophularia nodosa	x	-	-
Veronica chameadrys	x	-	-

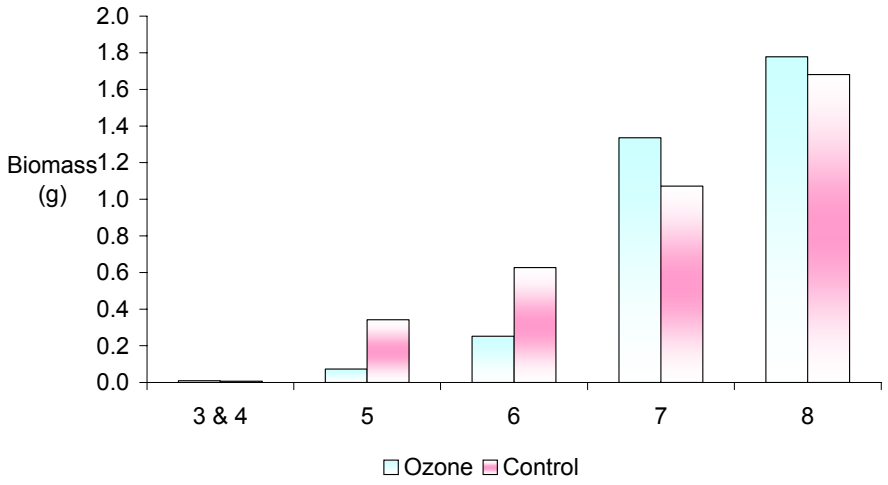
The results above are for the whole emergent woodland community or for individual species. Since species composition in the replicate mesocosms varied greatly, we used the Ellenberg index for light to assess the ecological patterns in the results. The Ellenberg index assigns a value to each species on a scale of 1 (deep shade) to 9 (open habitats), based on their realised (ecological) range. The data from each experiment for cover and biomass was analysed separately for each Ellenberg index. Table 1.5 shows the results for final biomass; data for cover showed similar trends.

Table 1.5: Summary of analysis of variance of results for final above-ground biomass in Experiments 1,2, and 3, by Ellenberg class. Arrows indicate a significant effect of ozome (P=0.05), ns indicates no significant of ozone and x indicates species group not present.

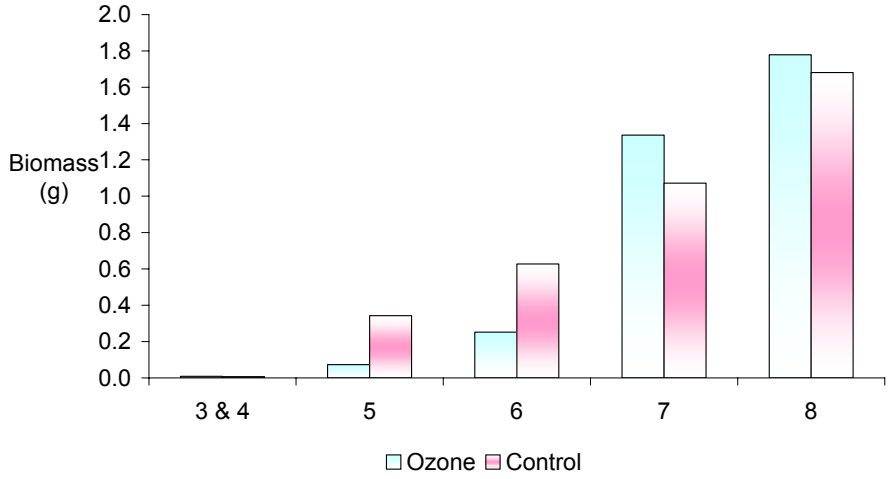
Ellenburg Rank	**1**	**2**	**3**
3 & 4	ns	ns	ns
5	ns	ns	ns
6	↓	ns	ns
7	ns	↓	ns
8	ns	x	ns

Overall few significant effects were found for individual Ellenberg classes, partly because of large experimental variability. However, the data do suggest systematic shifts in species composition between Ellenberg classes. Figure 1.4 shows a comparison from the two experiments (Experiment 1 and Experiment 2), in which significant effects were found by Ellenberg class. No consistent or significant effects of ozone on individual Ellenberg classes were found in Experiment 3.

The two experiments showed quite different patterns of response. In Experiment 1, biomass was dominated by species in Ellenberg groups 7 and 8; these are invasive, non-woodland species, the presence of which would decrease the conservation value of the woodland. The results show a decrease in biomass of Ellenberg groups 5 and 6 in ozone, whereas there was a non-significant increase in biomass (and to a greater extent cover) in groups 7 and 8. This implies that ozone caused a shift towards a community dominated by species that are less characteristic of woodland habitats.



(a). Experiment 1



(b). Experiment 2

Figure 1.4. Comparison of effects of ozone on above-ground biomass at the end of the experiment for different Ellenberg light classes (3-8) in Experiments 1 and 2

In Experiment 2, there was a greater contribution from characteristic woodland species (Ellenberg classes 3 and 4), whereas the proportion of biomass in Ellenberg groups 7 and 8 was much reduced. Ozone caused a decrease in biomass in all groups (except group 5 which was only a very small contribution) and there was no overall shift between Ellenberg groups. It should be noted (a) that total above-ground production in Experiment 1 was four times greater than in Experiment 2 in the control treatment and (b) that ozone had a significant overall effect on above-ground biomass in Experiment 2 but not in Experiment 1.

1.4 Conclusions

The three experiments carried out clearly demonstrated the sensitivity of many woodland species to ozone, as illustrated by the degree of non-specific and specific symptoms that appeared. Several species showed major reductions in growth following visible symptoms, and in some cases died off in a particular mesocosm. The effects of ozone had contrasting effects on community characteristics in the three experiments. In Experiment 3, the degree of experimental variability unfortunately precluded any

ecological interpretation, and therefore the key conclusions presented here focus on Experiments 1 and 2. These experiments were carried out in closed chamber systems; although irradiance levels are low and typical of those experienced by woodland ground flora, such experiments cannot be predictive of real impacts of ozone under field conditions. Nevertheless, the potential significance of the key findings in this part of the project can be summarised as follows, in relation to the original hypotheses:-

- There were effects of ozone on species richness and two key biodiversity indices, suggesting that ozone can affect the species composition of woodland ground flora significantly.
- The degree of shade did not significantly or consistently modify the impacts of ozone.
- In both experiments, the species most adapted to shade conditions (Ellenberg groups 5/6 in Expt. 1, groups 3/4 in Expt. 2) were adversely affected by ozone in terms of cover and above-ground biomass.
- In Experiment 1, in which soil was taken from a relatively open site with a high proportion of group 7 and 8 species, total biomass was not reduced, and there was an indication that the more invasive species benefited in the presence of ozone.
- In contrast, in Experiment 2, in which the soil was taken from a coppice site, total biomass was reduced; there were fewer invasive species, the level of competition was low, and the most abundant species present in Ellenberg groups 6 and 7 were all relatively sensitive to ozone.

In summary, the experiments confirm the implication from the experiments described in Section 2 that plant communities of upland woodland habitats may be relatively sensitive to ozone. The adverse effects of ozone were particularly marked on the more characteristic woodland species, but the overall effect on community structure may depend on the micro-habitat within the woodland. At open clearing or edge sites, the outcome of competition may be modified by ozone to favour invasive species. However, in denser shade, with less competition, it is the direct effect of ozone on the long-term viability of populations of woodland species that may be of greater significance.

1.5 Field observations and studies

1.5.1 Introduction

The laboratory experiments described in Sections 2 and 3 demonstrate the potential for ozone to affect the autecology and community composition of woodland and grassland communities in the uplands. However, the extent to which ozone actually affects these communities in the field depends on a number of factors, including how the position of

the species within the plant canopy modifies both the ozone concentrations to which it is exposed, and the stomatal conductance, which may influence the flux of ozone to sites of damage in the leaf. For example, lower ozone concentrations and reduced conductance might be experienced by woodland ground flora, because of ozone deposition and light interception by the woodland canopy. This may reduce its impact under field conditions. In order to further investigate these phenomena, field measurements were carried out during the summer of 2005 at two sites, the woodland site at Grass Wood and the grassland site at Colt Park.

1.5.2 Visible injury

It had originally been planned to carry out field surveys of injury symptoms on species found to exhibit typical ozone symptoms in the laboratory studies. However, as described in Sections 2 and 3, although accelerated senescence and chlorosis was common, few species (a total of six in the two experiments) consistently showed characteristic ozone symptoms. Furthermore, the summer of 2005 had relatively low concentrations of ozone in the study region. Thus, although no symptoms of visible injury which might be attributable to ozone were observed in the field, this should not be taken to indicate that such symptoms do not occur.

1.5.3 Within-canopy ozone concentrations

Measurements of within-canopy ozone concentrations were made with two portable ozone monitors. Two cross calibrated portable 2b ozone monitors were used to monitor levels of ozone. At each of the locations, one monitor was deployed to provide reference concentrations (outside the wood at Grass Wood; above the canopy at Colt Park), while the other was deployed to provide concentrations either within the wood or within the grass canopy. At Colt Park, the ozone concentration above the canopy was measured at the height of the vegetation (approximately 60cm.) and the ozone within the canopy was measured just above ground level (approximately 3-5cm above the ground). At Grass Wood, the 'inside' the woodland measurements were taken at approximately 4-5cm above the ground level and 'outside' the wood at 2m.

Measurements were made on a total of 5 days at Grass Wood and 2 days at Colt Park. The broad features of the data were similar on the different days at the two sites. Therefore, we present only one typical dataset from the two sites to illustrate the major features of the data.

Figure 1.5 shows data collected on 8-13 June 2005 at Grass Wood. There are interesting trends in the diurnal pattern of differences between outside and within wood concentrations. During the middle of the day, when ozone concentrations peaked, the concentrations in the wood were, as expected, lower than those outside. Concentrations inside the wood were typically reduced by 10-40%, the variation between days probably

relating to meteorological conditions. At certain occasions during the night, in contrast, concentrations were actually higher inside the wood than outside. It appears that one cause of this is a slower rate of decline of ozone concentrations in the wood during the late afternoon and evening, possible because of reduced atmospheric dispersion or reduced deposition rates at the lower wind-speeds inside the wood.

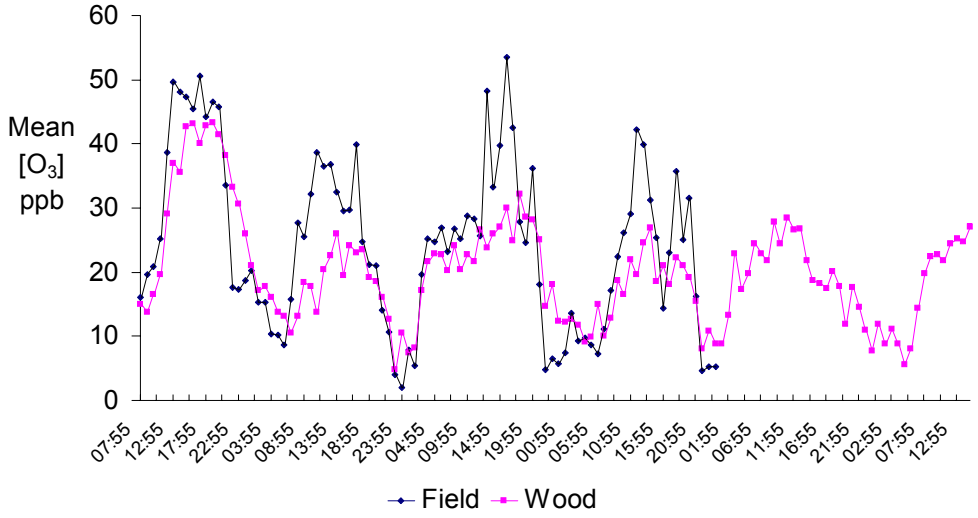


Figure 1.5. Measurements of ozone concentrations inside and outside a coppice area of Grass Wood over the period 8-13 June 2005.

Figure 1.6 shows data collected on 22-23 June 2005 at Colt Park. In contrast to the Grass Wood data, this covers only a period of one day. However, the data shows a strong reduction of concentrations within the canopy, with concentrations within the canopy being only 20-30% of those at the top of the canopy during the middle of the day. The effect was greater during the daytime than at night, suggesting that ozone uptake through the stomata may be important factor controlling ozone penetration to lower levels of the canopy.

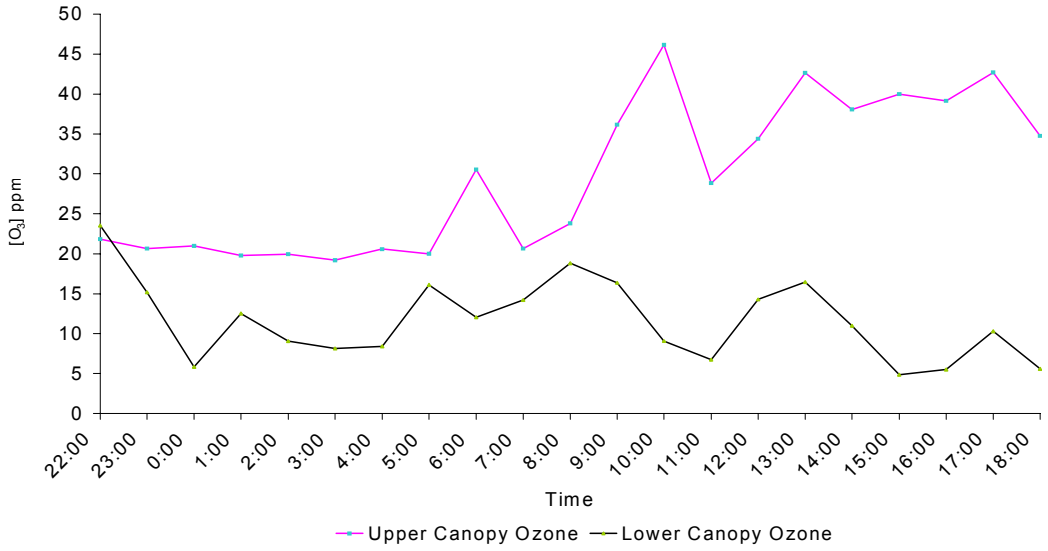


Figure 1.6. Measurements of ozone concentrations above and within a grassland canopy at Colt Park over the period 22-23 June 2005.

1.5.4 Stomatal conductance

Measurements of CO_2 and water vapour exchange of individual leaves were made at the Grass Wood and Colt Park sites. A Cirus-1 Infra-red Gas analyser was used for measurements of photosynthesis and conductance. At Grass Wood, measurements were made on typical woodland species, including *Allium ursinum*, *Viola riviana* and *Mercuralis perrennis*. At Colt Park, the measurements focussed on marsh marigold (*Caltha palustris*), because this allowed a comparison with measurements made within the project by Newcastle University.

At Grass Wood, the stomatal conductance of all species remained low throughout the day. This reflected the very low penetration of light into the woodland canopy. There was no evidence that short periods of increased photon flux density related to sunflecks were associated with increased stomatal conductance, although short-term increases in photosynthetic rate were observed in such periods.

At Colt Park, photon flux density at the bottom of the canopy varied over the course of the day, possibly reflecting variation in the measurement position. However, the results suggest, based on typical light response curves, that photon flux density would not be a significant factor limiting ozone stomatal conductance at the bottom of the canopy. This is broadly consistent with the measurements of stomatal conductance for *Caltha palustris*, which show little overall difference in stomatal conductance between the top and the bottom of the canopy.

1.5.5 Conclusions

Time limitations, and equipment failures in spring and late summer, reduced the amount of data obtained in this part of the study; because only a small number of days of data in mid-summer were available, interpretation of results must be made with caution. Nevertheless, the following points which arose from the work may be significant and need further investigation:-

- At the grassland site, position in the canopy did not have a major impact on stomatal conductance, but ozone concentrations were much lower at the bottom of the canopy. Hence inter- and intra-specific variation in ozone flux as a function of canopy position may be most dependent on the processes controlling ozone penetration through, and loss within, the dense grassland canopy in mid-summer.
- At the woodland site, ozone concentration was reduced, but to a smaller extent than was found in the grassland canopy. This may reflect the relatively high degree of air mixing into a small upland wood on a steep slope. The measured stomatal conductance was low and would likely be limiting to ozone flux. It would be important to assess the situation during the rapid spring growth period of

these woodland species, when tree canopy modification of both irradiance and ozone concentration may be lower.

1.6 Ozone effects on mire communities

1.6.1 Introduction and aims

The closed chamber fumigation system used for this study is not appropriate for multi-year studies of ozone impacts on vegetation, because of the artificial growth conditions. In order to carry out longer-term studies to assess the effects of elevated ozone on both plant community composition and ecosystem processes, we initiated a new mesocosm study in open top chambers (OTCs), in collaboration with Newcastle University. This community was selected because (a) there is little data available on responses of typical mire species; (b) mires are of conservation importance in the UK; and (c) reports from Finnish studies show that exposure to ozone might have short-term effects on methane emission and ecosystem respiration in mire communities, thus providing a potential feedback to global change.

The aims were to assess the effects of elevated ozone concentrations on a mire community, including changes in abundance, growth and chemical composition of major plant species, and fluxes of methane and CO_2.

1.6.2 Methods

Mesocosms were collected from a raised bog which is part of Roudsea Wood and Mosses at the northern shore of Morecambe Bay, Cumbria. The vegetation is classified as an M18 *Erica tetralix* - *Sphagnum papillosum* raised mire community. Intact mesocosms (diameter= 24 cm, length= 35 cm) were cored in PVC tubes and placed in buckets with deionised water to maintain water levels similar to mean values at the site (10 and 5 cm below the moss surface during the growing season (April-September) and winter, respectively). The vegetation is dominated by the peat moss (*Sphagnum papillosum*) and cotton sedge (*Eriophorum vaginatum*). Ozone exposure of the mesocosms was conducted in eight OTCs, two cores in each chamber. The elevated ozone treatment in four OTCs consisted of an ozone concentration of 75 ppb above ambient during the growing season (April-September) and an increased level of 10 ppb above ambient during winter. The control treatment in the other OTCs received non-filtered air.

Sphagnum length increment (modification of the cranked wire method), *Eriophorum* leaf density and stomatal conductance (August-October), methane emissions and ecosystem respiration (using an enclosure technique), soil temperature at 9 and 17.5 cm below the moss surface, were measured bi(monthly), while several other plant parameters were measured at the end of the growing season (and in June).

1.6.3 Results

Elevated ozone tended to have a negative impact on length increment of Sphagnum in summer, although the response was not significant (Figure 5.1). The ozone effect became more apparent in winter when the ozone level was only increased by 10 ppb at the high ozone treatment. This may have resulted from carry-over effects of the high ozone concentrations during the growing season or by the higher impact of elevated ozone on plant performance at lower temperatures. Both types of ozone responses have been observed for other plant species by the projects of CEH Bangor and Newcastle University, respectively. *Eriophorum* leaf density and stomatal conductance were not influenced by enhanced ozone exposure.

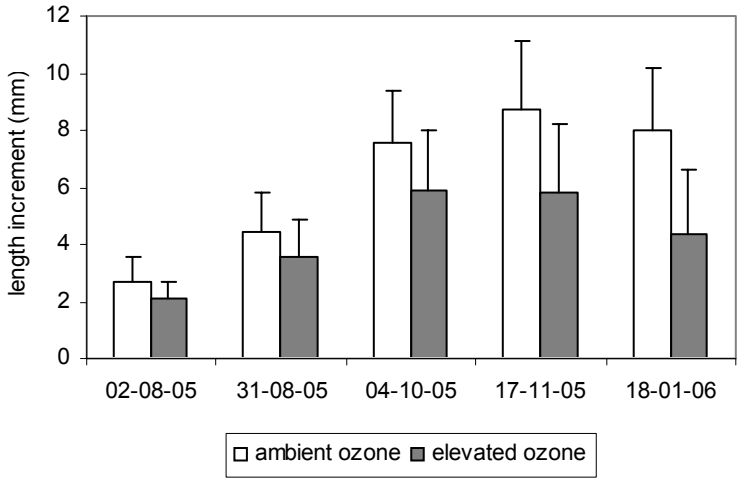


Figure 1.7 Length increment of *Sphagnum papillosum* in mire mesocosms at ambient (non-filtered air) and elevated ozone (ambient +75 ppb during the growing season and ambient +10 ppb in winter) after 27 (2nd August 2005) to 196 (18th January 2006) days of ozone exposure. Start of ozone exposure at 10th May 2005, start of length increments measurements at 6th July 2005. Data are means ± SE (n= 8).

Methane emissions were significantly reduced by elevated ozone during the growing season ($p=0.019$, Figure 5.2a). This ozone response did not occur any more during the first part of the winter period, which may be related to the lower added ozone concentration in winter. Ecosystem respiration was not affected by the ozone increase, except for the measurements on 31st August, when respiration was significantly greater at the high ozone exposure treatment (Figure 1.8b).

1.6.4 Conclusions

Although there is some evidence of effects of ozone after less than one year of exposure, we consider that continuation of the experiment for a further 2-3 years is essential to evaluate longer-term effects on this mire community. Detailed studies on the underlying mechanisms that cause these ozone responses will help to improve our understanding of how increases in ozone concentrations may influence plant communities and the carbon budget of mire ecosystems in the future.

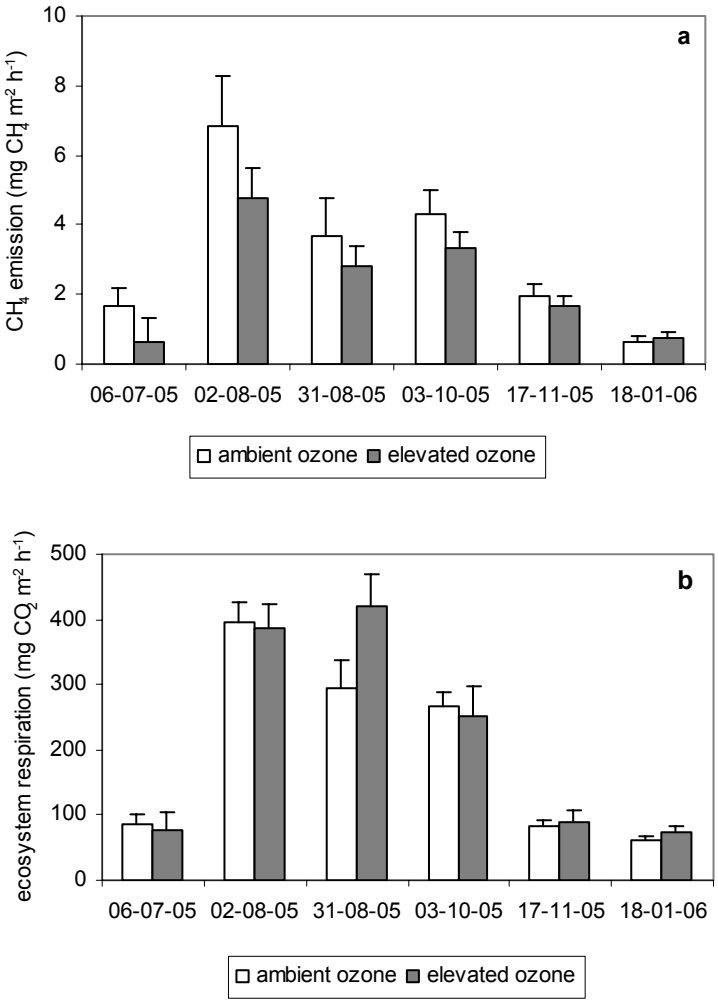


Figure 1.8 Methane emission (a) and ecosystem respiration (b) of mire mesocosms at ambient (non-filtered air) and elevated ozone (ambient +75 ppb during the growing season and ambient +10 ppb in winter) after 57 (6th July 2005) to 253 days (18th January 2006) of ozone exposure. Data are means ± SE (n= 8).

2 Effects of Ozone on UK Upland Vegetation

CEH Bangor: F Hayes, G Mills, P Williams, H Harmens and L Jones

Summary

At CEH Bangor, investigations into the effects of ozone on individual species and simulated communities of upland vegetation have been conducted by exposing plants to ozone in the solardomes (eight dome-shaped greenhouses). In 2003, thirty-three species from Snowdonia, North Wales, UK, were exposed for ten weeks to a weekly episodic ozone regime in solardomes representing predicted future concentrations. Two solardomes were used as controls, with ozone added to charcoal filtered air to give a continuous ozone concentration of 30 ppb (O3(30)). A weekly episodic ozone regime was applied to two other solardomes, with concentrations rising for 8 hours per day to 80 ppb on day 1, 100 ppb on days 2 and 3, and 80 ppb on day 4; ozone concentrations remained at 30 ppb at all other times (O3(30+peaks)). The control and background ozone concentrations of 30 ppb were maintained throughout the night as well as during the daytime. Over half of the species tested responded in one or more ways to the episodic ozone regime. Effects noted were ozone-specific leaf injury symptoms (e.g. Carex echinata) and/or premature senescence (e.g. Festuca rubra) and/or changes in above-ground biomass (e.g. Armeria maritima), whereas other species (e.g Holcus lanatus and Carex demissa) showed no effects. Some species, although showing no effects during the 10-week ozone exposure, showed carry over effects on biomass the following spring, after a winter period of ambient ozone exposure, (e.g. Galium saxatile, Nardus stricta and Saxifraga stellaris). Such carry-over effects indicate the potential ecological impact of ozone on semi-natural vegetation species and indicate the importance of longer-term studies on the effects of ozone on plants.

In 2004 and 2005, a two-year study was undertaken into the impact of increasing background ozone, with and without peaks, on mesocosms of a grassland community typically found in western and northern UK uplands (NVC U4: *Festuca ovina-Agrostis capillaris-Galium saxatile* grassland). The mesocosms contained seven species that were grown from plants collected in North Wales (UK) or from locally sourced seed: *Festuca ovina, Agrostis capillaris, Galium saxatile, Anthoxanthum odoratum, Potentilla erecta, Carex bigelowii* and *Carex echinata*. Ozone exposure was for 12 weeks each in 2004 and 2005, starting in July, and comprised LL: Low background (20ppb), low peaks (25ppb); LH: Low background (20ppb), high peaks (75ppb); HL: High background (45ppb), low peaks (50ppb) and HH: High background (45ppb), high peaks (100ppb). 'High peaks' of ozone were applied for four consecutive days in each seven-day period to provide an episodic ozone regime. Increasing the background ozone concentration from 20-25 ppb (LL treatment) to 45-50 ppb (HL treatment) did not affect the biomass of the

communities, but did result in enhanced senescence. In the HH treatment, senescence was well established in several species by the end of the 2005 season, and a significant reduction in biomass was found in the grass species *A.odoratum*. Visible injury symptoms were not detected on the plants grown within the communities even though an ozone exposure experiment conducted in the solardomes in 2003 with an AOT40 of 18.3 ppm.h, approximately half that of the HH treatment in 2004, resulted in injury on *C. echinata* and *P. erecta*. It seems likely that a lower absorbed dose occurred when these, small, low-growing plants were exposed to ozone as part of a dense-canopy community. This may also partially explain why reductions in biomass in *A. odoratum* were only detected when harvest material was combined for the three harvests, and not in the first year of exposure as would have been expected from the single species pot experiments conducted in 2003.

In addition, a community representing ***Festuca ovina-Agrostis capillaris-Thymus praecox*** grassland (NVC CG10) was exposed to ozone for one season in 2005. Increased senescence was observed in the highest ozone treatments on *Festuca ovina, Agrostis capillaris* and *Viola riviniana*. The percentage cover of *Agrostis capillaris* was decreased in the high background-high peaks treatments, but there was no significant effect on biomass. There were also no significant effects on biomass of any of the component species or on mesocosm biomass.

2.1 Introduction

Ozone pollution has long been known to damage vegetation, and concentrations of ozone in many parts of Europe exceed the current Critical Levels for ozone (LRTAP Convention, 2004). Several studies have shown that some sensitive vegetation species are affected at ambient ozone concentrations in Europe (e.g. Bergmann et al., 1995; Power and Ashmore, 2002; Gimeno et al*.,* 2004, Pleijel and Danielsson, 1997). Ambient ozone concentrations are predicted to rise over the next 50 years (Coyle *et al.*, 2003). This is thought to be of particular importance in rural mountainous areas such as Snowdonia (North Wales, UK), where the current daylight mean ozone concentrations are already close to 40 ppb, the threshold used in calculations of AOT40 (the sum of the differences between the hourly mean ozone concentration (in ppb) and 40 ppb when the concentration exceeds 40 ppb during daylight hours, accumulated over a stated time period). It is also predicted that annual average ozone concentrations in the majority of the UK will rise to about 40 ppb by 2030 (Coyle et al., 2003). The ambient ozone concentrations of rural mountainous areas tend to have a higher background level than comparative urban areas (Coyle et al*.,* 2002) and the night-time ozone concentrations of rural mountainous regions can remain high.

Prior to this study, only a small proportion of the semi-natural vegetation species of the UK have been tested for sensitivity to ambient ozone pollution (Davison and Barnes, 1998). Of the species tested, many show effects of ozone including ozone-specific visible injury (e.g. Bungener et al*.,* 1999a), early senescence (Bergmann et al., 1995; Franzaring et al*.,* 2000) and alterations in biomass and root:shoot biomass partitioning (e.g. Gimeno et al., 2004). There is also evidence that natural selection has occurred for some species to favour those genotypes with increased ozone resistance, e.g for *Plantago major* (Lyons et al*.,*1997). Few studies have investigated carry-over effects of ozone exposure. Where these have been investigated, this has usually been to consider the effects of ozone treatment in a consecutive growing period, e.g. Fumagalli et al. (2003) and Soja et al. (1997). Carry-over effects in the absence of additional ozone treatment have not been reported.

The aim of the first part of the study was to test common upland plants found in Snowdonia for sensitivity to ozone and to consider the ecological significance of the responses identified. In 2003, 33 species were exposed to ozone within individual pots, whilst in 2004 and 2005, simulated communities representing two vegetation types typically found in western and northern UK uplands, NVC U4 (*Festuca ovina-Agrostis capillaris-Galium saxatile* grassland) and CG10 (*Festuca ovina-Agrostis capillaris-Thymus praecox* grassland) were exposed to ozone for two and one season respectively. Exposure to ozone was in computer-controlled solardomes, with simulated episodic regimes representing current and future ozone climates. In addition to measuring the effects of ozone on plants during and immediately following exposure to peak episodes of ozone, this study also investigated the ability of a species to re-grow in the following spring to establish whether there were carry over effects from ozone exposure that may affect the long-term survival of upland plants.

The following two papers have been submitted resulting from this study, and are in press:

Hayes, F., Mills, G., Williams, P., Harmens, H., Büker, P. Impacts of summer ozone exposure on the growth and overwintering of UK upland vegetation. Atmospheric Environment, in press.

Mills, G., Hayes, F., Williams, P, Jones, M.L.M., Macmillan, R., Harmens, H., Lloyd, A., Büker, P. 2005. Should the effects of increasing background ozone concentration on semi-natural vegetation communities be taken into account in revising the critical level? Background paper workshop on 'Critical levels of ozone: further applying and developing the flux-based concept', 15-19 November 2005, Obergurgl, Austria.

2.2 Effects of ozone on individual species

2.2.1 Methods

Plants were collected from an NVC CG10-12 intermediate community (*Festuca ovina-Agrostis capillaris-Thymus praecox* grassland; *Festuca ovina-Alchemilla alpina-Silene acaulis* dwarf herb community, Rodwell et al., 1992) in Snowdonia, North Wales, UK, where this community is widespread. Twelve plants per species were collected from Clogwyn Coch (SH 603555) and Cwm Idwal (SH 640597) in May and June 2003, with assistance and permission from the Countryside Council for Wales (CCW), and potted into acidic compost mix (40 litres ericaceous compost mixed with 25 litres sharp sand), calcareous compost mix (3 parts John Innes compost No2 mixed with 1 part gritty sand and 1 part peat) or multi-purpose compost as appropriate for each species. Plants were raised in sheltered outdoor conditions. In addition, the following grass species were grown from seed (Emorsgate Seeds, Norfolk, UK): *Agrostis capillaris, Agrostis vineale, Holcus lanatus, Festuca rubra* and *Festuca ovina.* Final selection of species for exposure was determined by the availability of sufficient healthy plants after collection and propagation; the species used are shown in Table 2.1.

Table 2.1: Species included in this study.

Forbs	*Grasses*
Arabis petraea	*Agrostis capillaris*
Armeria maritima	*Agrostis vinealis*
Campanula rotundifolia	*Anthoxanthum odoratum*
Cirsium arvense	*Festuca ovina*
Cryptogamma crispa	*Festuca rubra*
Dryas octopetala	*Holcus lanatus*
Eriophorum angustifolium	*Nardus stricta*
Galium saxatile	
Juncus effusus	
Juncus squarrosus	
Narthecium ossifragum	
Oxalis acetosella	*Sedges*
Plantago lanceolata	*Carex demissa*
Potentilla erecta	*Carex echinata*
Oxyria digyna	*Carex laevigata*
Ranunculus acris	*Carex panicea*
Rumex acetosella	
Saxifraga stellaris	
Scirpus cespitosus	
Senecio fluviatilis	
Thymus polytrichus	
Viola lutea	

In late July, the 12 plants per species were numbered from 1 (smallest) to 12 (largest). These were then divided into three size classes (1-4, 5-8 and 9-12), and one individual plant per size class was randomly selected for each of the four solardomes. The grasses

Holcus lanatus, Agrostis capillaris, Agrostis vinealis and *Anthoxanthum odoratum* were cut back to 7cm. *Festuca ovina, Festuca rubra* and *Nardus stricta* were not cut back as they were less than 7cm tall. Nutrients were provided to these grass species after they were cut back ('phostrogen', PBI Home and Garden, UK). Plants were sprayed to control aphids during the exposure period using the commercial pesticides 'Provada' and 'Bifenthrin'. Plants were transferred to the solardomes on 31st July and exposure to ozone started on 1st August.

Plants were exposed in solardomes (hemispherical glass domes 2m high and 3m diameter), each receiving approximately 2 air changes per minute (Figure 2.1). Ozone was generated by passing oxygen through an ozone generator (Wallace and Tiernan) and a computer-controlled (LabVIEW, version 6) mass-flow controller system was used to deliver ozone to the solardomes. Two solardomes were used as controls, with ozone added to charcoal filtered air to give a continuous total ozone concentration of 30 ppb (O_3(30)). A weekly episodic ozone regime was applied to two other domes, with concentrations rising for 8 hours per day to 80 ppb on day 1, 100 ppb on days 2 and 3, and 80 ppb on day 4; ozone concentrations remained at 30 ppb at all other times (O_3(30+peaks)). Ozone exposure was for 10 weeks (1st August to 6th October). Ozone concentrations in the solardomes were monitored on a 30-minute cycle using an ozone analyser (Envirotech API400). The total ozone dose was an AOT40 of 18.3 ppm.h in the O_3(30+peaks) domes, and below 0.01 ppm.h in the O_3(30) domes. The difference in ozone dose between the two O_3(30+peaks) domes was less than 2%.

Figure 2.1: CEH Bangor solardome facility

All plants were assessed weekly for ozone injury and senescence. A leaf was classified as having ozone injury if it had any ozone-specific injury symptoms, visible as white or yellow stipples on the leaf surface, and was classified as senesced if 25% or more of the leaf was senesced, otherwise it was classified as healthy. After 4 and 8 weeks of

exposure to ozone, most grass species were cut back to 7cm (*Holcus lanatus, Agrostis capillaris, Agrostis vineale, Festuca ovina, Festuca rubra* and *Anthoxanthum odoratum*). These biomasses were added to the biomass from the final harvest to give a total biomass per species at the 10-week harvest. *Nardus stricta* was not cut back due to its low stature (material above 7 cm was predominantly flowers). After 10 weeks of exposure to ozone all plants were harvested to soil level. Above-ground biomass was removed and dried at 65°C for a minimum of 7 days. For the species *Carex echinata, Carex demissa, Carex panicea, Carex laevigata* and *Nardus stricta* the above-ground biomass was sorted into green and senesced (>25% of the leaf senesced) leaf material and flowers.

After harvesting to soil level, the pots were overwintered in solardomes with non-filtered air and ambient ozone exposure only. The fans were not running so the air-flow was low. All plants of each species were overwintered in the same solardome. Plants were watered by hand as necessary and in May 2004, plants were harvested to soil level. Above-ground biomass was dried at 65°C for a minimum of 7 days.

Values from the three plants per species per dome were averaged to provide two replicates per treatment prior to statistical analysis. The effects of ozone were assessed by one-way ANOVA using Minitab (version 14) using plant size as a covariate.

2.2.2 Results

Ozone-specific leaf injury was observed on *Potentilla erecta, Carex echinata, Dryas octopetala, Oxalis acetosella, Nardus stricta, Eriophorum angustifolium*, *Carex panicea* and *Carex demissa* (data not presented). For the majority of these species the injury was observed within two weeks of the start of ozone exposure. *Carex echinata* plants exposed to the O_3(30+peaks) treatment showed ozone-specific injury symptoms on a few leaves per plant, equating to approximately 1% of leaves after 3 weeks. The extent of ozone injury increased to approximately 2% of leaves affected by the end of the 10-week exposure. *Potentilla erecta* plants exposed to the O_3(30+peaks) treatment showed ozone-specific injury symptoms on 4% of leaves after 3 weeks, 9.5 % of leaves after 6 weeks and on 6% of leaves after 10 weeks. *Nardus stricta* showed no visible injury symptoms before week 10 of the exposure period, when injury was observed on a few leaves of one plant in each replicate solardome receiving the O_3(30+peaks) treatment.

Increased / premature senescence of plants exposed to the O_3(30+peaks) regime was observed during the weekly assessments for several species, including *Scirpus cespitosus, Viola lutea, Agrostis capillaris, Agrostis vineale* and *Anthoxanthum odoratum* (Figures 2.2 and 2.3). Differences in the extent of senescence of some of these species in the O_3(30+peaks) treatment compared to the O_3(30) treatment occurred within a few weeks for the majority of these. Premature senescence was particularly noticeable in

Anthoxanthum odoratum, where after exposure for 4 weeks, mean senescence of O_3(30+peaks) treated plants was 12.8%, compared to 4.1% for O_3(30) treated plants ($P<0.01$). The extent of senescence at each weekly assessment was used to compare the rate of development of senescence and those species that showed a significant increase in the rate of development of senescence in the O_3(30+peaks) treatment compared to the O_3(30) are shown in Figure 2.2. These species were *Anthoxanthum odoratum* ($p<0.05$), *Agrostis capillaris* ($p<0.05$), *Festuca rubra* ($p<0.01$) and *Oxalis acetosella* ($p<0.05$). For *Festuca rubra* and *Oxalis acetosella* there was a very large increase in the extent of senescence in the O_3(30+peaks) and O_3(30) treated plants towards the end of the ozone exposure, although there was very little senescence and no difference in the extent of senescence between the two treatments until after six and eight weeks of exposure respectively. Both *Agrostis capillaris* and *Anthoxanthum odoratum* showed senescence and differences in the extent of senescence between treatments much earlier in the study. There were reductions in extent of senescence between some weeks for these species during the course of the exposure as the plants continued to produce new leaves throughout the exposure period.

Approximately one third of the species tested showed a significant increase, or a strong trend for an increase, in the extent of senescence at the end of the ten week exposure with the O_3(30+peaks) treatment (Figure 2.3). The species with significant differences in the extent of senescence were *Carex echinata* ($p<0.01$), *Anthoxanthum odoratum*, *Agrostis vinealis*, *Arabis petraea*, *Festuca rubra* and *Festuca ovina* (all with $p<0.05$). Trends for an increase in senescence with ozone exposure ($p<0.1$) were also shown for *Carex demissa, Juncus squarrosus, Saxifraga stellaris* and *Armeria maritima.* An increase in the rate of development of senescence over the exposure period had only been detected for a few of these species.

The leaves of several species were sorted into green and senesced (where >25% of the leaf was senesced) at the final harvest. There was a significant increase in the dry weight of senesced material for *Carex echinata* ($p<0.01$) and *Carex laevigata* ($p<0.05$) and a trend for *Carex panicea* ($p=0.055$) plants that were treated with the episodic ozone treatment compared to the O_3(30) control (Figure 2.4). There was also a large but not statistically significant increase in the senesced biomass of O_3(30+peaks) treated *Carex demissa.* There were no differences in the extent of senesced material in O_3(30+peaks) compared to O_3(30) treated *Nardus stricta.*

Shoot biomasses for species with significant changes due to the episodic ozone treatment, or changes of >20% (reduction or stimulation) are shown in Figure 2.5. Significant decreases in above-ground biomass in response to the episodic ozone exposure were observed for *Armeria maritima, Nardus stricta* and *Scirpus cespitosus*.

Several species (*Festuca rubra, Festuca ovina, Juncus effusus*) showed large (>20%) reductions in above-ground biomass of O_3(30+peaks) treated plants compared to O_3(30) treated plants which were not statistically significant due to high plant-plant variability. In addition, some species (*Plantago lanceolata, Senecio fluviatilis, Juncus squarrosus, Thymus polytrichus, Potentilla erecta*) showed an increase in biomass of >20% with episodic ozone exposure, but none of these increases in response to ozone were statistically significant.

Several species that had been treated with O_3(30+peaks) in 2003 showed large shoot biomass reductions compared to O_3(30) treated plants in spring 2004, even though there had been no ozone treatment during this overwintering period. These reductions were significant for *Juncus effusus* (33%, $p<0.05$) and *Saxifraga stellaris* (97% $p<0.05$; Figure 2.6). Large, but not significant, above-ground biomass reductions occurred for *Carex laevigata, Carex echinata, Galium saxatile* and *Nardus stricta*. Most of the above-ground biomass reduction in *Carex echinata* was due to a large reduction (30%; $p<0.001$) in flower biomass in O_3(30+peaks) treated (mean flower biomass 0.67g) compared to O_3(30) treated plants (mean flower biomass 0.99g). In contrast, significantly higher above-ground biomass of the O_3(30+peaks) treated plants compared to control plants was measured for *Senecio fluviatilis* ($p<0.05$) and *Agrostis capillaris* ($p<0.01$), and also a large but not significant increase for *Juncus squarrosus* (Figure 5). Some species did not regrow after being cut back to soil level (e.g. *Armeria maritima*).

Fourteen of the species in this study showed no significant responses to the episodic ozone exposure in terms of visible injury, increased/premature senescence or above-ground biomass at the end of the exposure. These were *Holcus lanatus, Campanula rotundifolia, Carex demissa, Cirsium arvense, Cryptogamma crispa, Galium saxatile, Juncus squarrosus, Narthecium ossifragum, Oxalis acetosella, Oxyria digyna, Plantago lanceolata, Ranunculus acris, Rumex acetosa* and *Thymus polytrichus.* Some of these species re-grew following overwintering with ambient ozone exposure but showed no significant differences in regrowth between those which had been exposed to of O_3(30+peaks) the previous summer and those exposed to the O_3(30) control.

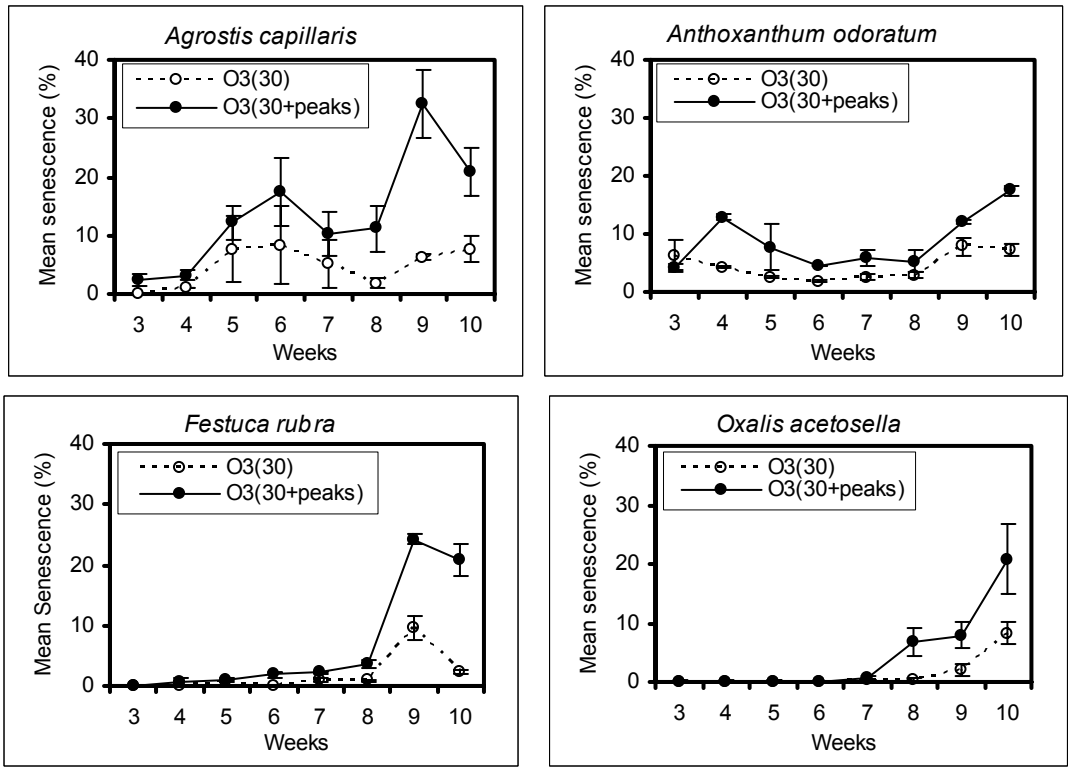


Figure 2.2: Development of senescence in $O_3(30)$ and $O_3(30+peaks)$ treated plants during exposure to the ozone regime for selected species, where 'Weeks' is the number of weeks since the start of the exposure. Bars are standard errors.

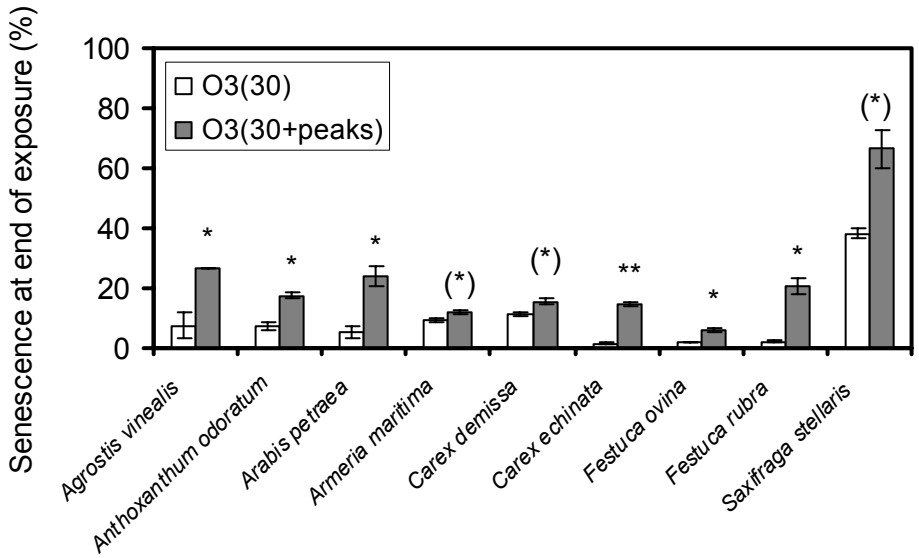


Figure 2.3: Extent of senescence at the end of the exposure for those species that showed large and/or significant differences between $O_3(30)$ and $O_3(30+peaks)$ treated plants. Bars are standard errors. * and ** indicates significant treatment effects at $p<0.05$ and 0.01 respectively, (*) represents a significant difference at $p<0.1$.

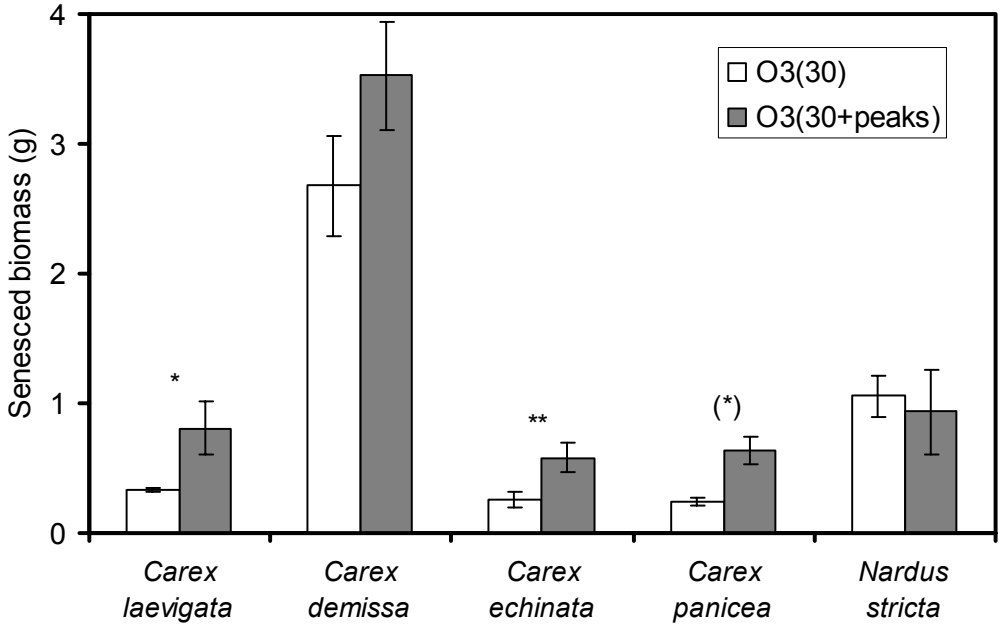


Figure 2.4: Biomass of senesced leaf material of species at the final harvest after exposure to $O_3(30)$ or $O_3(30+peaks)$. Bars are standard errors. * and ** indicates significant treatment effects at $p<0.05$ and 0.01 respectively, (*) represents a significant difference at $p<0.1$.

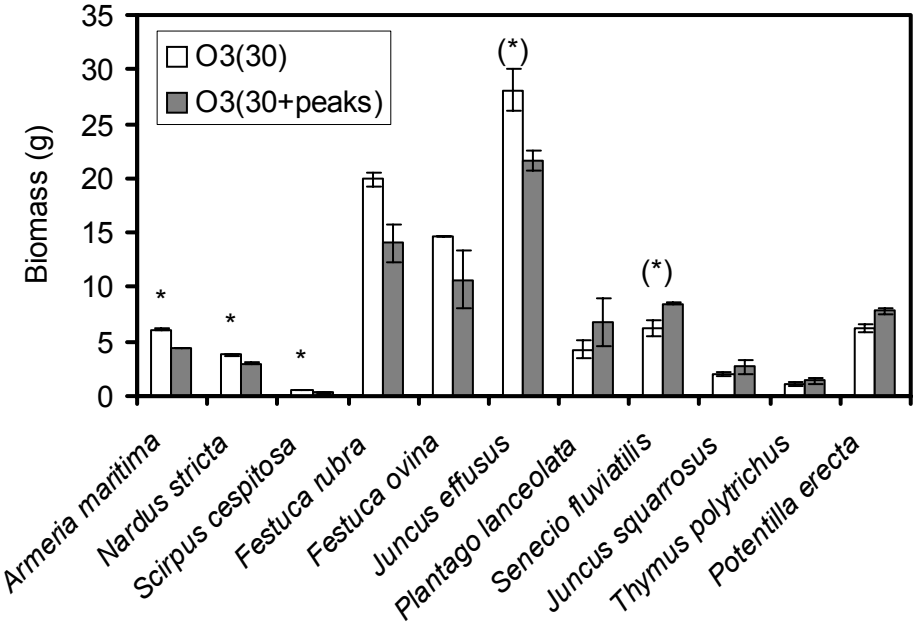


Figure 2.5: Biomass at the end of the ozone exposure for those species with significant and/or large differences between $O_3(30)$ and $O_3(30+peaks)$ treated plants. Bars are standard errors. * indicates significant treatment effects at $p<0.05$, (*) represents a significant difference at $p<0.1$.

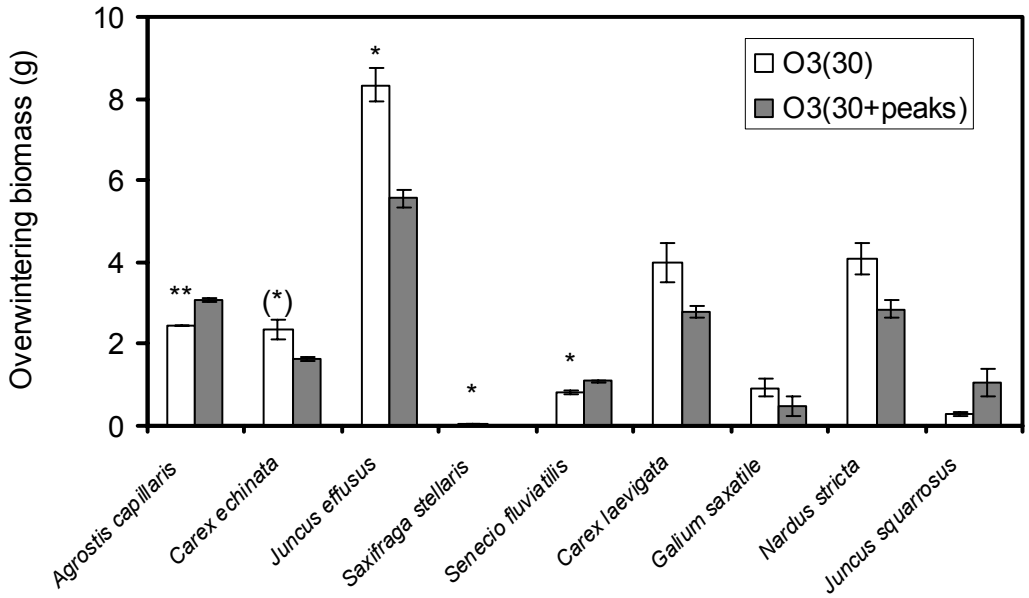


Figure 2.6: Biomass in spring 2004 for those species with significant and/or large differences between plants exposed to O_3(30) and O_3(30+peaks) treatments in the previous summer. Bars are standard errors. * and ** indicates significant treatment effects at $p<0.05$ and 0.01 respectively, (*) represents a significant difference at $p<0.1$.

2.3 Effects of ozone on simulated communities

2.3.1 Ozone exposure

Contract EPG … together with additional funding from NERC supported the addition of four further ozone-exposure solardomes to the facility during the winter of 2003/04. The computer–control system was also upgraded, allowing four treatments with two replicate solardomes per treatment. The treatments applied using a computer controlled system were: LL: Low background (20ppb), low peaks (25ppb); LH: Low background (20ppb), high peaks (75ppb); HL: High background (45ppb), low peaks (50ppb) and HH: High background (45ppb), high peaks (100ppb). 'High peaks' of ozone were applied for 4 consecutive days in each 7-day period to provide an episodic ozone regime (Figure 2.7). The system was further upgraded during the winter and spring of 2004/05 to include new oxygen and ozone generators, new safety O_3 detectors in each dome to prevent over-exposure and an improved Labview control system. The actual backgrounds, peak values and AOT40s I the domes are indicated in Table 2.2. AOT40 values in 2005 were lower than in 2004 reflecting the reduced values for the "high" background in that year to below 40 ppb. Ozone exposure was from 14th July to 5th October in 2004 and 8th July to 27th September in 2005.

Table 2.2: Ozone concentrations and AOT40 values in the solardomes

	2004			2005		
Treatment	Background (ppb)	Peak (ppb)	AOT40 (ppm h)	Background (ppb)	Peak (ppb)	AOT40 (ppm h)
Low Low	20.3		0	17.2		0
Low High	20.3	70.6	16.0	18.1	68.8	10.8
High Low	48.7		13.3	37.1		4.2
High High	47.6	97.1	36.2	39.8	93.7	23.6

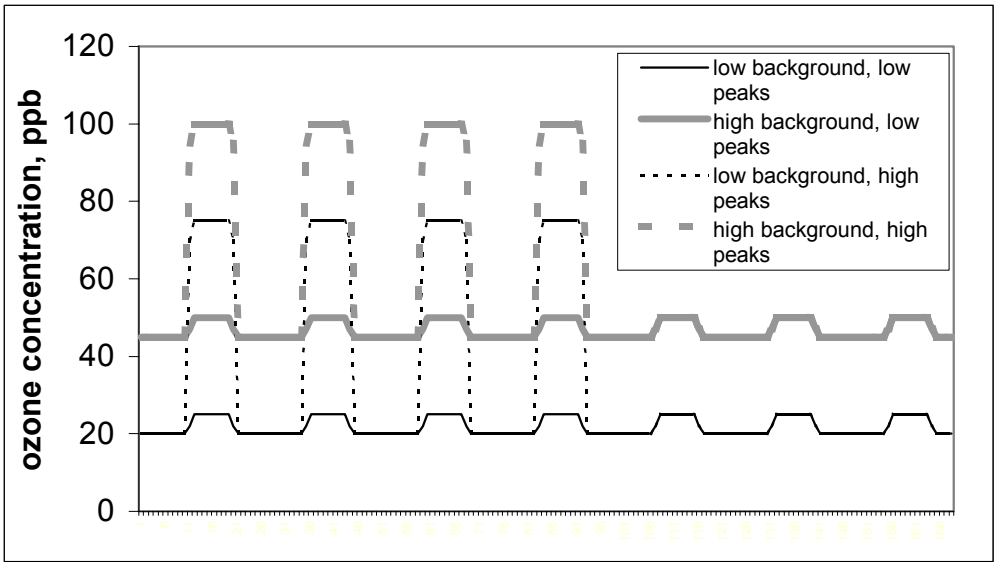


Figure 2.7: Weekly target ozone regime in 2004 and 2005.

2.3.2 Effects of ozone on a U4 simulated community exposed for two consecutive summers (2004 and 2005)

2.3.2.1 Method

Plants of *Anthoxanthum odoratum, Carex echinata, Carex bigelowii, Potentilla erecta* and *Galium saxatile* representing those found in a UK upland habitat (National Vegetation Classification U4, *Festuca ovina-Agrostis capillaris-Galium saxatile* grassland, equivalent to EUNIS community E1.72) were propagated from stock plants that originated from the Snowdonia National Park in North Wales, UK. *Festuca ovina* and *Agrostis capillaris* were sown from seed obtained from a commercial seed supplier (Emorsgate, UK). Eight weeks prior to exposure to ozone, established plants were transferred to straight-sided containers (24cm diameter, 11cm deep) filled with a mixture of ericaceous compost and sharp sand in a ratio of 40 litres to 25 Kg. These model communities each had the same arrangement of species in each pot and were established in a cool greenhouse for 8 weeks. The compost mixture of each community was treated with the systemic pesticide imidochloprid. A few days prior to exposure, the vegetation was cut back to 11 cm. All vegetation growing outside the pot perimeter was also removed. Five replicate pots were placed in each of eight solardomes at the start of the 12 week exposure on 14th July, 2004 (Figure 2.8).

Unfortunately, in the middle of the 2004 season a system fault resulted in loss of one of the HH treatments. A second set of pots exposed in the other HH dome originally planned for use in studying biomass effects on different layers in the canopy (data not discussed here) was harvested as indicated, and the pot numbers were randomly allocated into two HH blocks. In the following year, these two blocks of pots were placed in each of the two HH solardomes, allowing replication to be continued in the two season experiment. All pots were over-wintered at an outdoor, but sheltered site. The second season of ozone exposure started on 8th July, 2005 and again lasted for 12 weeks.

Figure 2.8: Simulated U4 community on day 1 of exposure

Communities were watered during the early morning (4 a.m.) using an automated misting system. Communities were checked frequently to ensure that all communities were watered sufficiently.

Ozone visible leaf injury and senescence were recorded as the percentage of leaves showing either type of damage on 25% or more of the leaf surface. Whole communities were also measured fortnightly using a spectroradiometer (PPSystems) and the reflected light at specific wavelengths was used to determine green biomass and carotenoid/chlorophyll content.

All community plant canopies were all cut back to 7cm at the end of the first ozone exposure period, and biomass of the component species determined. The communities were then left to overwinter in sheltered but outdoor conditions. A weak nutrient solution was applied every eight weeks over the winter period.

The percentage cover of each species was determined in early March, 2005. Canopies were cut down to 7cm in June 2005 and the harvested vegetation was sorted into the component species prior to drying in an oven at 65°C. The communities were

transferred to the solardomes on 4th July to acclimatise before ozone treatments re-started on 8th July. After eleven weeks of exposure to the ozone regime in year 2 the percentage cover of each of the component species in each community was determined. After a total of twelve weeks exposure to the ozone regime in year 2 the percentage cover was again determined and the communities were harvested to soil level and sorted into the component species prior to drying in an oven at 65°C and weighing.

Values from the five simulated U4 communities per dome were averaged to provide two replicates per treatment prior to statistical analysis. The effects of ozone were assessed by ANOVA using Minitab (version 14) with plant size as a covariate.

2.3.2.2 Results

No ozone-specific visible injury was observed on any of the component species in any treatment during either of the twelve-week exposures of the communities to ozone. However, senescence was observed on *Agrostis capillaris, Anthoxanthum odoratum, Festuca ovina, Potentilla erecta* and *Carex bigelowii* during the course of the exposure periods in both year 1 and year 2. The extent of senescence was highest in the high-background-high peaks treatments in all cases. There was more senescence in all treatments (including the low background-low peaks) in 2005 compared to 2004, however there were no differences in relative sensitivity to ozone between species or treatments in the second season of exposure compared to the first. As there was increased senescence in the low background-low peaks treatment in 2005, the mean senescence in the LL treatment was subtracted from the values for the other treatments to standardise the effects due to ozone exposure. An example for *Festuca ovina* in the high background-high peaks treatment is shown in Figure 2.9.

There was a trend for an increased ratio of carotenoid to chlorophyll content in the high background-high peaks treatment compared to the other treatments after exposure to the ozone regime for six weeks (data not presented), however this was not significant at the $p<0.05$ level. The ratio of carotenoid to chlorophyll content was further increased in the high background-high peaks treatment compared to the other treatments after eleven weeks of ozone exposure (data not presented). This increase was significantly different ($p<0.05$). Again differences in the carotenoid to chlorophyll ratio between the other treatments were not significant.

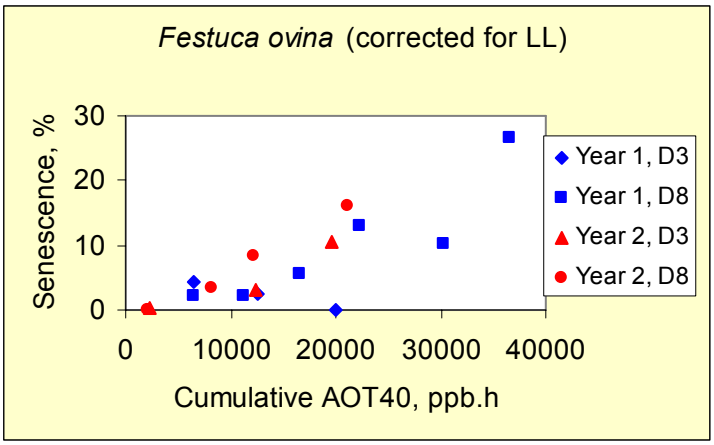


Figure 2.9: Comparison of senescence in response to ozone dose for *Festuca ovina* in the high background-high peaks treatments in year 1 and year 2. In each case, the values have been corrected according to the senescence in the low background-low peaks treatment during that week.

There were no significant effects of ozone treatment on the biomass of individual component species or total biomass after exposure to ozone in 2004, after overwintering until spring 2005 or after exposure to ozone during summer 2005 (data not presented). However, the cumulative biomass of *Anthoxanthum odoratum* was significantly reduced ($P<0.05$) by approximately 15% in the high-background-high peaks treatment compared to the other treatments (Figure 2.10). This coincided with a decrease in the percentage cover of *Anthoxanthum odoratum,* which although showing no significant differences after exposure to ozone in 2004 or after overwintering in spring 2005, showed differences between treatments after exposure to ozone in 2005 (Figure 2.11). The cover of *Anthoxanthum odoratum* was reduced from 53% in the low background-low peaks treatment, to 42% in the high background-high peaks treatment, although this difference was not significant due to the high variation between replicates.

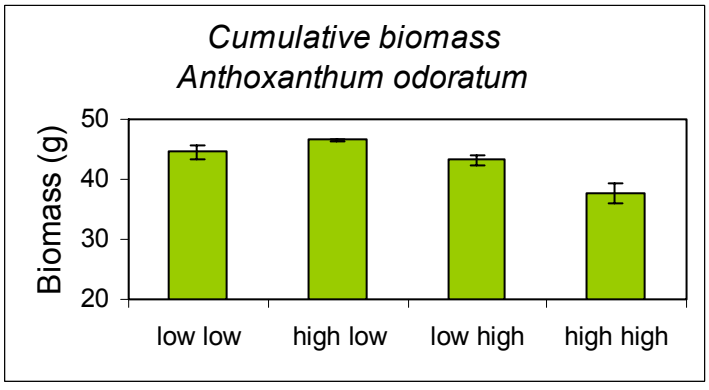


Figure 2.10: Cumulative biomass of *Anthoxanthum odoratum* exposed to the ozone regime for two consecutive summers. Bars are standard errors.

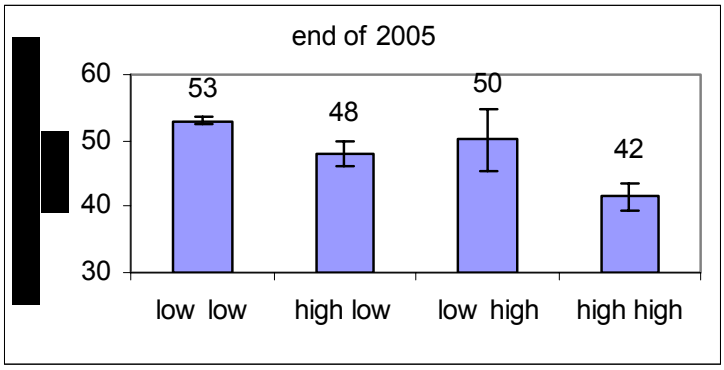


Figure 2.11: Percentage cover of *Anthoxanthum odoratum* at the end of 2005, after exposure to the ozone regime for 2 consecutive summers. Bars are standard errors.

Senescence of the community was increased with increasing background ozone concentration in the absence of additional peaks of ozone (Figure 2.12). One of the species contributing to this difference was *Festuca ovina,* which had significantly accelerated development of senescence in the high background – low peaks ozone treatment (P = 0.012) compared to the low background – low peaks treatment in the exposure during year 1 (data not presented). In 2005, this difference was not as pronounced, however, the difference in mean ozone concentration between the two treatments was also not as large in 2005 as in 2004. There was also a strong trend for increased development of senescence in the high background – low peaks treatment compared to the low background – low peaks for *Potentilla erecta* in year 1 (P = 0.066, data not presented). Differences between treatments in the rate of development of senescence were not significant in either year for *Agrostis capillaris, Anthoxanthum odoratum* or *Carex bigelowii*

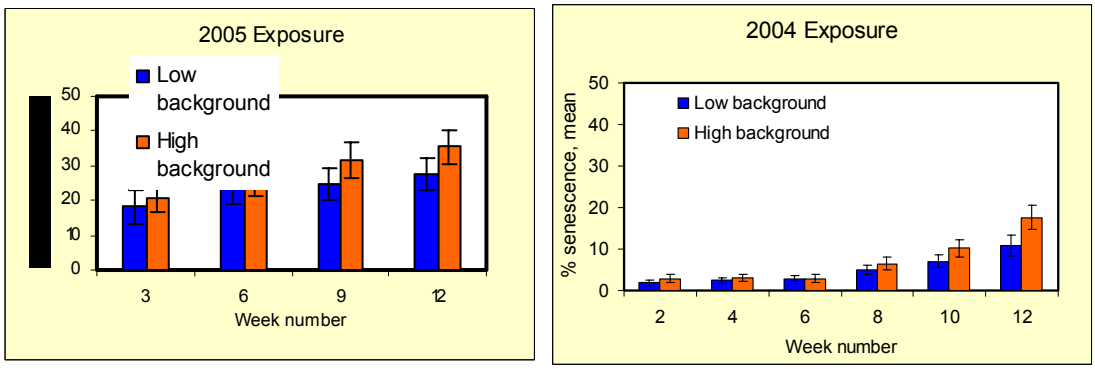


Figure 2.12: Mesocosm senescence with increasing background ozone concentration in 2004 and 2005. Bars are standard errors.

There were subtle changes in community composition with high background ozone (low peaks) concentrations compared to low background (low peaks) ozone (Figure 2.13). With increased background ozone concentration, the proportion of *Agrostis capillaris* was increased and the proportion of *Festuca ovina* was decreased.

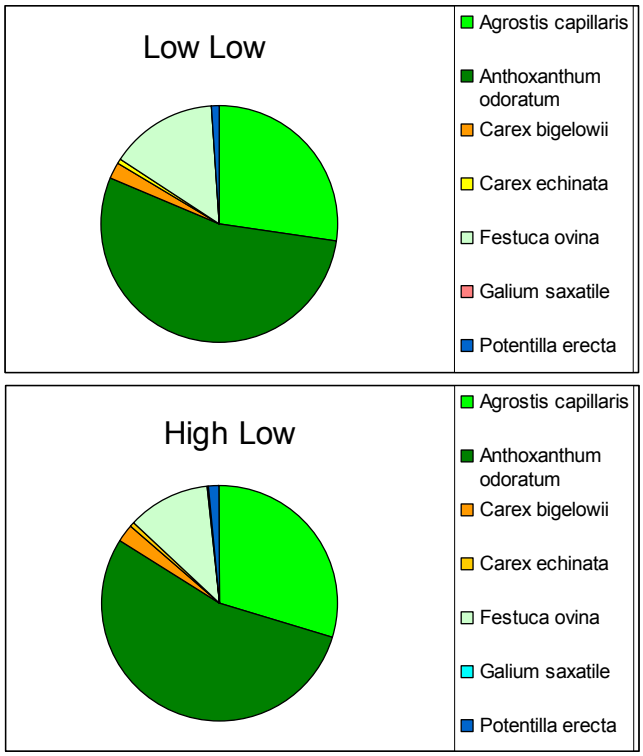


Figure 2.13: The proportions of the component species in the mesocosms after exposure to the ozone regime for two consecutive summers. The treatments are low background-low peaks (low low), and high background-low peaks (high low).

When peaks of ozone were present, increase in background ozone concentration also corresponded with an increase in senescence of the mesocosms (Figure 2.14). There were also changes in the proportions of the component species of the mesocosms, with increasing background ozone corresponding with a small increase in the proportion of *Agrostis capillaris* and a small decrease in the proportion of *Anthoxanthum odoratum* (data not presented).

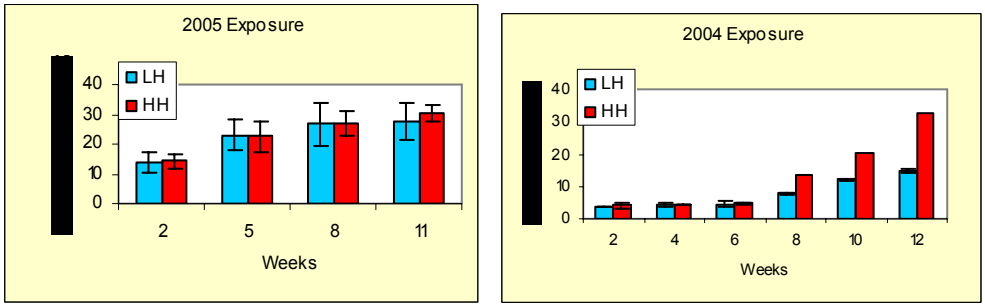


Figure 2.14: Mesocosm senescence with 'low background-high peaks' and 'high background-high peaks' ozone treatments in 2004 and 2005, where 'Weeks' is the number of weeks from the start of that exposure period. Bars are standard errors.

2.3.3 Effects of ozone on a CG10 model community exposed for one season (2005)

2.3.3.1 Method

Plants of *Agrostis capillaris, Festuca ovina, Viola lutea, Viola riviniana, Thymus polytrichus, Campanula rotundifolia, Prunella vulgaris* and *Potentilla erecta* representing

those found in the UK upland habitat *Festuca ovina-Agrostis capillaris-Thymus praecox* grassland (NVC CG10) were established first as individuals, then eight weeks before exposure to ozone started established plants were transferred to straight-sided containers (24cm diameter, 11cm deep) filled with a mixture of ericaceous compost and sharp sand in a ratio of 40 litres to 25 Kg. Communities were established in a cool greenhouse for eight weeks, then five replicate pots were placed in each of the eight solardomes on 8th July, 2005 for 12 weeks (Figure 2.15).

Figure 2.15: Simulated CG10 communities prior to the start of exposure

2.3.3.2 Results

No visible injury due to ozone was observed on any of the component species during the course of the experiment. Premature senescence was observed on *Festuca ovina, Agrostis capillaris* and *Viola riviniana*. At the final harvest there was significantly more senescence on *Agrostis capillaris* from the high-background-high peaks treatment compared to the other treatments ($p<0.05$). There was also a large but not significant increase in senescence of *Festuca ovina* in the high background-high peaks treatment. Senescence per mesocosm was 15% higher in the high background-high peaks treatment, but this difference was not significant.

The percentage cover of *Agrostis capillaris* was decreased in the high background-high peaks treatments (Figure 2.16, $p<0.1$), but there was no significant effect on biomass. There were also no significant effects on biomass of any of the component species or on mesocosm biomass.

2.4 Discussion

This study has indicated that over half of the upland vegetation species tested were affected by episodic ozone exposure, with sensitive species showing a combination of earlier senescence, visible injury, biomass reduction and/or reduced over-wintering. When six or seven species were grown together as a simulated community, effects of

ozone were less pronounced and took longer to appear. Visible injury was virtually absent, and biomass reductions were only detected during the second year of exposure.

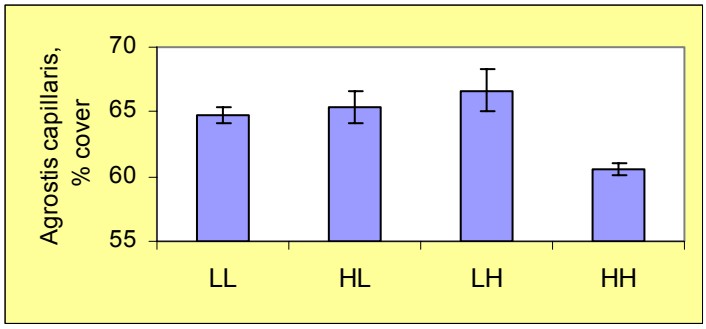


Figure 2.16: Percentage cover of *Agrostis capillaris* after exposure to the ozone regime for twelve weeks. Bars are standard errors.

When grown singularly, visible ozone injury was observed within only two weeks of ozone exposure for some species, e.g. *Potentilla erecta, Oxalis acetosella* and *Carex echinata*. This was a relatively quick onset of ozone-specific visible injury, which implies that these species may be particularly sensitive to ozone. In some wetland species, visible injury has also been found to develop quickly, with three of the twelve studied species showing visible injury symptoms within 10 days of exposure to ozone (Power and Ashmore, 2002). In the current study only about half of the species which had visible injury showed a significant reduction in above-ground biomass at the end of the ten-week exposure. *Dryas octopetala, Potentilla erecta, Oxalis acetosella, Eriophorum angustifolium* showed no other effects of ozone exposure, so visible injury may not equate to an ecological impact for these species. This was also shown in some herbs and grasses by Pleijel and Danielsson (1997) where injury was not always accompanied by reduced biomass or decreased reproductive output.

Many species grown singularly in this study showed increased senescence in the episodic ozone treatment, but only a few of these showed a reduction in above-ground biomass. In contrast, *Armeria maritima* showed a reduction in above-ground biomass when no other symptoms had been observed. Biomass reductions in the absence of any other symptoms have previously been reported in other species, e.g. some subalpine species (Mortensen and Nilsen, 1992) and *Plantago major* and *Plantago coronopsis* (Reiling and Davison, 1992). A possible explanation for a decreased biomass with no other symptoms is that the plants may be allocating resources for protection and repair against oxidative stress. For example, dark respiration was increased by ozone exposure in *Plantago major* (Zheng et al., 2000) and in a later study Zheng et al. (2002) demonstrated that the amount of the photosynthetic molecule Rubisco was reduced by ozone exposure.

Several species including *Juncus squarrosus, Senecio fluviatilis* and *Plantago lanceolata* when grown singularly showed increased above-ground biomass after exposure ozone.

Stimulations of shoot growth in response to ozone have been previously shown in a few other species, e.g. *Molinea caerulea* (Franzaring et al., 2000), *Bromus erectus* and *Silene dioicea* (Bungener et al., 1999b), although the mechanisms for this are unclear. It is possible that root:shoot ratios have been altered in favour of the shoot to compensate for the loss of effective leaf area by ozone. This has been shown in species such as *Plantago lanceolata* and *Desmazeria rigidia* (Reiling and Davison, 1992) and *Lathyrus pratensis* (Power and Ashmore, 2002). It was not possible to investigate root biomass in this study to leave the roots intact so that plants could re-grow over winter to investigate carry-over effects of ozone. Furthermore, growth of a second set of plants, purely for the purposes of assessing root biomass, was not possible due to constraints both from space within the solardomes and availability of plant material because these plants were collected from the Snowdonia National Park under strict guidance and limitations on the number of specimens of any one species from the Countryside Council for Wales.

Interestingly, three species (*Galium saxatile, Nardus stricta* and *Saxifraga stellaris)* showed reductions in above-ground biomass following over-wintering with no additional ozone exposure, even though no effects on above-ground biomass or senescence had been observed during the ozone exposure or at the final harvest. The response of these species to ozone has not been studied in detail before, but it is possible that changes in biomass partitioning occurred due to reduced allocation of resources to the roots. Decreased storage of plant reserves in the roots during the overwintering period would impede the ability of the plant to regrow in spring. A reduction in allocation to the roots due to ozone exposure has previously been shown in other species e.g. *Cirsium arvense* (Power and Ashmore, 2002) and several wetland species (Batty and Ashmore, 2003). In addition, Franzaring et al. (2000) showed a reduction in the root:shoot ratio of *Cirsium dissectum* after exposure to ozone for 28 days. In contrast, a few species exposed to O_3(30+peaks) in 2003 showed increased above-ground biomass in spring 2004 compared to O_3(30) treated plants. Possibly there was an increase in root allocation in these species following ozone exposure, as has previously been shown for *Lolium perenne* and *Rumex acetosella* (Reiling and Davison, 1992), which may have increased the ability of the plants for regrowth.

Carry-over effects have been shown previously in a few other perennial species. Soja et al. (2003) found that grapevines exposed to ozone showed reductions in grape yield, which were better related to the ozone exposure in the two years prior to harvest than when only considering the final year of ozone exposure. Measurements of chlorophyll fluorescence also indicated an increased susceptibility to ozone during the second year (Soja et al., 1997). Carry-over effects have also been shown for white clover (Fumagalli et al., 2003; Nussbaum et al., 1995), where regrowth in subsequent ozone-exposure periods was affected whilst the biomass at the end of the ozone exposure was not

affected. Similar effects have been found in this study, where some of the largest differences in biomass between treatments after overwintering were observed for species that had shown no effects during the exposure period (e.g. *Galium saxatile* and *Juncus effusus*). This could again possibly be due to increased use of plant resources for defence and repair mechanisms during the growing season at the expense of allocation of carbon to roots in plants exposed to peak levels of ozone.

Experiments as part of the EU-funded BIOSTRESS project have shown that early season ozone exposures of only 4-6 weeks may cause shifts in the species composition balance, and the effects of this may last for the rest of the growing season (Bender et al., 2002). Long-term effects, particularly those that persist into the following growing season, such as reduced regrowth after overwintering, will have the potential to affect the species balance of plant communities. In the current study, this hypothesis was tested in 2004 and 2005 by exposing the mesocosms of U4 grassland to two consecutive summer ozone regimes separated by an autumn/winter/spring exposure to ambient ozone and climatic conditions.

The mesocosm study has confirmed that the magnitude of the response of a species growing as part of a community is not always as predicted from individual component species. Work on individual species in 2003 suggested that there could be significant effects on many of the component species of the two model communities used in subsequent years. However, the magnitude of effects of ozone on species grown as part of a community was less than predicted and in some cases no effect of ozone was seen in the community even though effects were predicted following the experiments on individual species. Some studies suggest that the influence of the plant canopy on the microclimate can alter the stomatal conductance of the community component species. In addition, the ozone concentrations within a plant canopy may be reduced compared to the top or just above the canopy. These could combine to give different ozone fluxes to the different community species and may explain some of the discrepancies between predicted and observed responses to ozone in plant communities.

Although effects were fewer and smaller than expected, significant differences were observed between the different ozone treatments after exposure for two seasons. Slow responses in changes in biomass in response to ozone have also been demonstrated for some other communities (e.g. field –release exposure of hay-meadows; Volk et al., in press). Within a community, reductions in the biomass of an individual species may occur in the year(s) following enhanced and premature senescence due to ozone, as species that have senesced early may have a reduced capacity for regrowth in subsequent years. The increase in senescence shown in this study may indicate that community vitality has been affected by ozone exposure.

This study has indicated potential effects of increasing background concentrations of ozone. Significant differences between mesocosms of the model plant communities were shown when background ozone concentrations were increased both in the presence and absence of additional peaks of ozone representing ozone episodes. These results are important because background ozone concentrations are currently rising (Coyle et al., 2003) and results from these simulated communities suggest that significant effects of ozone on these relatively common grasslands of upland north-west UK could occur within the next 50 years. The screening experiments conducted in 2003 have indicated that where individual species are growing without intense intra- or inter-specific competition, several species including *Armeria maritima, Scirpus cespitosus, Nardus stricta, Anthoxanthum odoratum, Carex echinata, Juncus effusus, Saxifraga stellaris* and *Galium saxatile* are at significant risk of biomass reduction and reduced capacity for over-wintering. There is thus the potential for these species to decline in naturally occurring communities under repeated exposure to high ambient ozone concentration, as the competitive balance between species could possibly be altered.

2.5 Further work at CEH Bangor

Further information is required on the range of species potentially at risk from ozone. It is recommended that more species are screened for sensitivity to provide a reasonable representation of the species present in upland UK. These species should also be screened using an ozone profile currently representative of the UK uplands as well as predicted for the uplands in 2003, 2006 and 2090. This would facilitate the development of a response-matrix that would allow the third of the century in which species and the communities they represent are likely to respond to the changing ozone profile to be identified. It is also recommended that selected species are exposed to ozone as simple simulated communities to validate the predictions for plants growing in a competitive environment. Finally, stomatal conductance measurements should be made for species grown singularly and in competition, allowing flux-effect relationships to be established.

Acknowledgements

Defra and NERC are thanked for financial support of the studies conducted at CEH Bangor. In addition, P Hadfield (CEH, 2003 & 2004 seasons), Aled Jones and Peter Forster (IDB, 2005 season) are thanked for engineering support for the solardome system. P Büker (2003) and A Lloyd (2005) are thanked for providing technical support whilst Felicity Hayes was on maternity leave.

3 Mechanistic studies of antioxidants and their linkages with ozone sensitivity

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3.1 Principal aims

- Investigate the impacts of rising background concentrations of ozone on a targeted upland plant community of high conservation value
- Probe the importance of ozone uptake by leaves in deep shade/at night/ at lower temperatures i.e. situations where detoxification capability considered to be minimal
- Extend current understanding of the role of cell wall constituents in the capture and neutralization of ozone following it's dissolution in the leaf apoplast

3.2 Major findings:

- Conservative increases in ozone concentrations, as predicted for the UK over the next 30-50 years, resulted in significant shifts in the composition of a species-rich upland meadow community within a relatively short period (12-14 months to yield statistically discernible changes); with rare grasses of high conservation value (*Phleum* and *Briza*) suppressed in favour of an opportunistic grass (*Alopercurus*)
- Evidence of substantial night-time conductance in a significant fraction of the upland flora
- Exposure to ozone at night or at low temperature was shown to have a disproportionately adverse effect on plant growth, this may indicate a need to 'weight' ozone exposures (e.g. day/night and by season), when considering risk assessment approaches for upland vegetation

3.3 Principal outputs/outreach:

Significant UK dataset contributing to the refinement of critical exposures for the protection of long-lived plant communities from ozone pollution (via UNECE Workshop at Obergugl, Austria [2005] and ICP-Vegetation Workshop Bangor [2006]).

Data were presented as posters/verbal presentations at:

- UK Air Pollution Workshops in Newcastle (2004), York (2005) and Edinburgh (2006).
- UNECE Workshops in Harrogate (2003) and Obergugl (2005)
- ICP-Workshops in Poland (2005) and Bangor (2006)

The community-based investigations which made a significant UK contribution to the refinement of critical exposures for the protection of perennial herbaceous vegetation from ozone are reported in:

Bassin S, Volk M, Fuhrer J (2006) Predicting the sensitivity to ozone of temperate European grasslands: an overview. *Environmental Pollution*, In Press

3.4 Rationale

In upland and/or remote regions, diurnal and seasonal variations in ozone concentrations are much less pronounced than those experienced in lowland and/or urban areas. Although conductance commonly peaks in mid-afternoon for many species native to such situations, there are many observations in the literature that indicate that stomatal aperture is much less sensitive to environmental drivers than observed for lowland species and as a result there maybe significant uptake in upland situations at low light intensities and at night (due to incomplete stomatal closure). Indeed, many C_3 shrubs, trees, ferns and wetland species exhibit significance stomatal conductance at night, as do CAM plants (reviewed by Musselman & Minnick, 2000). This indicates that many species in upland situations maybe subject to significant rates of ozone uptake at low light intensities, at night and during spring/autumn when temperatures maybe low, under these conditions detoxification potential would be expected to be minimal (as detoxification processes are believed to be driven via photosynthetically-derived reductant and/or immediate photosynthetic products).

Vegetation responses to ozone are governed by the effective flux (*EF*) of the pollutant (and/or its reactive products) reaching the mesophyll plasmalemma (considered by the majority as the key biological target for ozone). As a consequence, at any given point in time (t), the effective flux into plant foliage represents the balance between ozone flux through the stomata (F) and the capacity of plant tissue to neutralize the incoming gas and/or it's reactive dissolution products (i.e. defensive capability, D)[1]. The need to incorporate plant defensive mechanisms within risk assessment procedures (such as mapping flux threshold exceedance and/or the setting of ambient air quality standards) is recognized (see 2002 Annual Report on DEFRA contract EPG/1/3173 and EPG/1/3/193), but plant-based defences are poorly understood and difficult to quantify – comprising elements that are both constitutive ('background levels/activity' of antioxidant metabolites/enzymes plus internal leaf geometry/structure) and inducible (shifts in defence-related gene expression and post-translational modification of proteins).

Recent work indicates that ascorbate (Vitamin C) plays a key role in constitutive defence against ozone and/or its reactive dissolution products. It is not known just how important apoplast ascorbate is in wild species – several observations suggest that in the field it

[1] Hence , cumulative effective ozone flux = $\int_0^T [F(t) - D(t)]dt$

may not be important – or whether upland species exhibit significant diurnal variations in the level of ascorbate in the leaf apoplast – some reports indicate that apoplast ascorbate levels undergo marked diurnal variations (Barnes *et al.* 2002) while others indicate that apoplast ascorbate levels maybe stable for prolonged periods in the dark (Pignocchi *et al*. 2002, Bass and Barnes, *in prep.*). The reason for these apparent discrepancies is unknown at this time – the observations could represent species-to-species variations in response or differences in the way in which such studies have been conducted.

3.5 Experimental Programme

A gifted student (Maria Samuelsson) was recruited from Gothenberg University, and she began work on the project 1st Sept. 2003. However, she developed serious health problems, and did remarkably well to deliver the experimental programme on target, in between hospitalization/operations and bouts of ill-health. Sadly, her health deteriorated to such an extent that she had to return to Sweden for a series of operations in January 2006. In order to progress the project in her absence, a well-qualified research associate (Dr. Simon Peacock) was appointed part-time to progress the work programme and deliver the experimental outcomes on target.

The work programme benefited from the unforeseen drop-down of 50 long-established mesocosms, containing an upland species-rich mesotrophic grassland community subject to predation by hay-rattle[2] (a hemi-parasite) and representing two levels of residual soil fertility. These mesocosms were established in 2000 during the course of the delivery of DEFRA Project BD1439, the principal outcomes of which were recently published in Nature (Bardgett *et al*., 2006). The availability of these mesocosms facilitated Newcastle-based open-top chamber studies on the impacts of predicted shifts in the upland ozone climate on a species-rich community of high conservation value and typical of the vegetation under threat in the Yorkshire Dales National Park.

The experimental programme delivered at Newcastle involved parallel studies on plant communities in the field (open-top chamber studies employing mesocosms) and individuals (mechanistic studies performed in controlled environment chambers).

3.5.1 Impacts of ozone on an established upland plant community of high conservation value

Species-rich mesocosms[3], established in 2000 and representing a factorial combination of two residual soil fertilities (high soil fertility [equivalent to ADAS phosphorus index 2: 16-

[2] *Rhinanthus minor*

[3] high-density polypropylene square-sectioned pots containing 42 dm^3 of subtrate (10 cm layer of limestone chippings in base of pot covered by an alluvial gley soil collected from two sites in Dentdale, North Yorkshire representative of contrasting past fertilization regimes (ADAS P-O; Field 5379; grid ref SD716868, latitude 54°16' N, longitude 2°26' W, situation: 138 m.a.s.l.. ADAS P-2; Field 0319 grid ref. SD730862, latitude 54o16' N, longitude 2°24' W, situation: 160 m.a.s.l) (for additional information see Peacock *et al*. 2004)

25 P_2O_5 mg l^{-1} or low soil fertility [equivalent to ADAS phosphorus index 0: 0-9 P_2O_5 mg l^{-1}]) and two hemi-parasite treatments (hay-rattle absent or hay-rattle present [60 plants m^{-2}]), were transferred from the field to four open-top chambers sited at Newcastle University's Field Station at Close House, Heddon-on-the-Wall, Northumberland in March 2004 where, following a period of establishment, they were exposed to a simulated present-day upland ozone climate versus a predicted 2050 upland ozone climate. These treatments equated to exposure to *c*. 30 ppb ozone (day and night) during summertime and 20 ppb (day/night during winter-time) in the present-day ozone treatment versus *c*. 50 ppb ozone (day and night) during summertime and 30 ppb (day/night during winter-time) in the 2050 ozone treatment (see Figure 3.1). Following the management prescription adopted since the creation of the mesocosms, the sward was cut to a height of 7 cm above the substrate on July 15th, and at monthly intervals thereafter. For the July harvest species' were separated and the oven-dry biomass of constituents recorded. At subsequent harvests the same year, forbs were separated from grasses and the dry weight of these fractions determined.

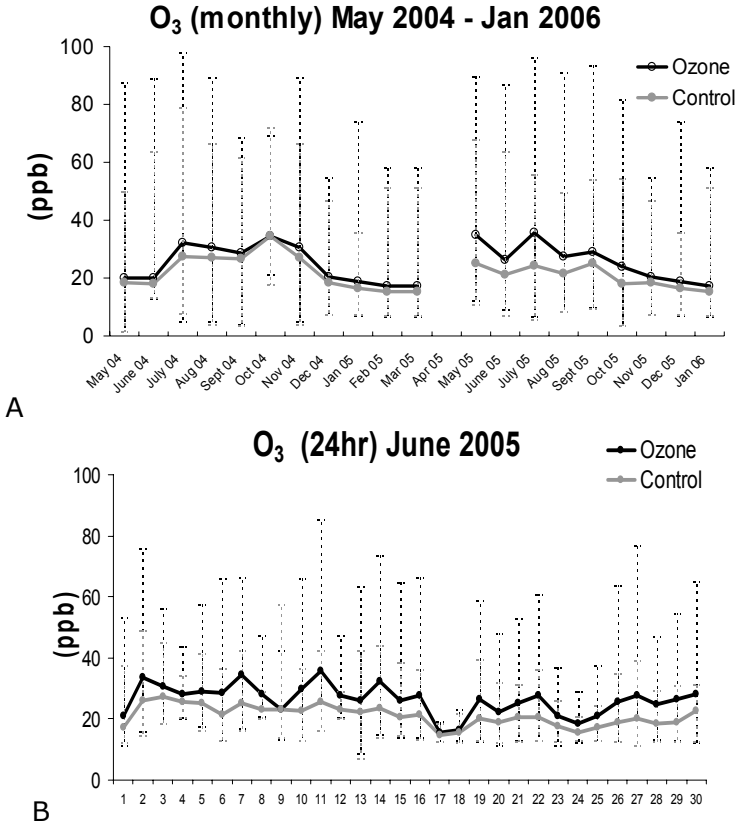


Figure 3.1. Ozone exposure summary for open-top chamber experiment. Plant communities were exposed to a simulated present-day upland ozone climate versus a predicted 2050 upland ozone climate. A. monthly mean ozone levels (± max/min concentrations)[4] B. representative hourly mean concentration profile over the course of the day (graph shows hourly mean concentrations for June 2005± max/min hourly mean concentrations over the course of the month).

[4] data unavailable between April and May 05 due to failure of datalogging system

Harvest data for 2005 revealed a substantial shift in composition of the mesocosms following transfer from the field to open-top chambers, with vigorous grasses (in particularly *Lolium*) benefiting from the switch in growing conditions (see Appendix 1). No significant effects of ozone were detectable during the first exposure season, but the substantial shifts in species composition induced by hay-rattle and residual soil fertility (reported previously by Smith *et al*., 2003) were maintained following transfer to open-top chambers (see Appendix 2 and 3). Interestingly, given the relatively low ozone exposures to which these plant communities were subjected, statistically significant ($P < 0.05$) effects of ozone on species composition were detectable at the harvest conducted in July 2005; exposure to a 2050 ozone climate resulting in a significant ($P < 0.05$) increase in *Alopercurus pratensis* at the expense of two of the rarer grasses in this mesophilic grassland community (and considered important from a conservation perspective in upland Britain), *Phleum bertolonii* and *Briza media* (see Figure 3.2). There were no significant interactions between ozone and residual soil fertility or hemi-parasitism.

The experiment will be continued for the foreseeable, with shifts in species composition monitored. The data resulting, contributed significantly to the revision of the critical exposure for the protection of long-lived natural/semi-natural plant communities at the UNECE Workshop in Obergugl, Austria (Nov. 2005).

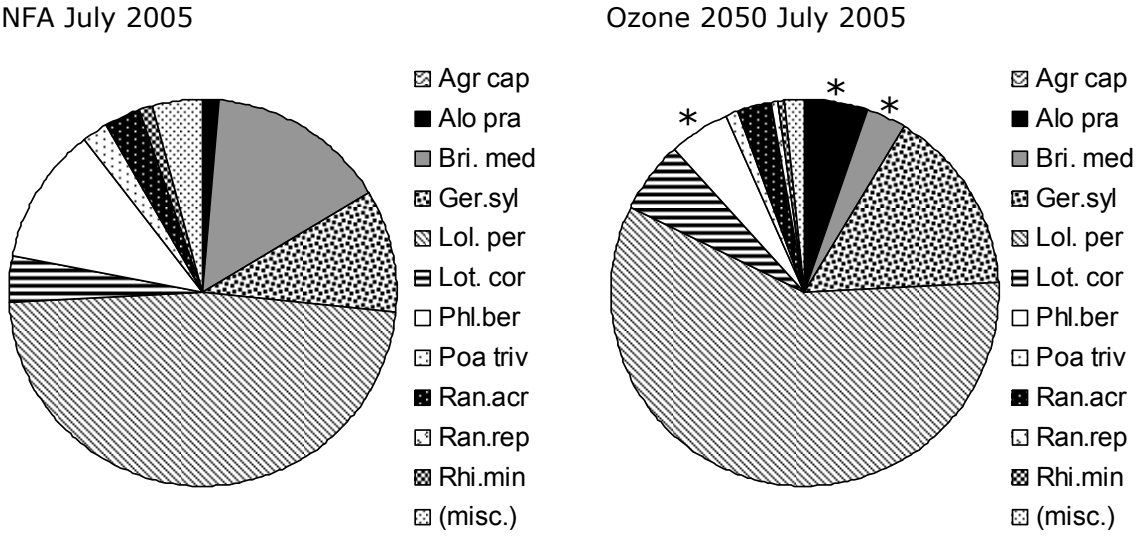


Figure 3.2. Impacts of 14 months' exposure of long-established species-rich mesophilic grassland communities to a simulated present-day upland ozone climate versus a predicted 2050 upland ozone climate. Significant shifts in *Alopercurus pratensis*, *Phleum bertolonii* and *Briza media* denoted by asterisks.

3.5.2 Factors governing impacts of ozone on upland plant communities: mechanistic studies on individual plants

3.5.2.1 Day/night ozone uptake in upland species

In upland and/or remote regions, diurnal and seasonal variations in ozone concentrations are much less pronounced than those experienced in lowland and/or urban areas. Although stomatal conductance, the major determinant of stomatal uptake into vegetation, commonly peaks in mid-afternoon for many species native to such situations, a meta analysis of the available literature reveals a significant fraction of herbaceous, as well as tree species, which exhibit substantial stomatal conductance at night-time – with many of these plants frequenting the uplands of Britain.

In order to provide additional information relevant to the identification of species exhibiting high night-time stomatal conductance which could be under threat from ozone in the field, as well as providing a useful experimental model for mechanistic studies, measurements were made with the aid of Delta-T porometers (i) in situ at a DEFRA upland field site located near Alston, Northumberland, and (ii) on pot-grown plants raised in controlled environment chambers.

These measurements revealed the expected variation in night-time stomatal conductance, with several species e.g. *Caltha palustris* and *Digitalis purpurea* exhibiting extremely high conductance at night, and some eg. *C. palustris* and *S. nodosa* exhibiting virtually the same conductance at night-time as during the day, both in the field and in controlled environment studies (see Figure 3.3)

The high night-time stomatal conductance exhibited by *Caltha* and *Digitalis*, in parallel with the capacity to extract apoplast fluid from the leaves of these species led to their selection as model plants for in-depth studies probing a range of factors influencing ozone sensitivity and linkages with antioxidative metabolism

3.5.2.2 Are night-time ozone fluxes more damaging than equivalent day-time flux?

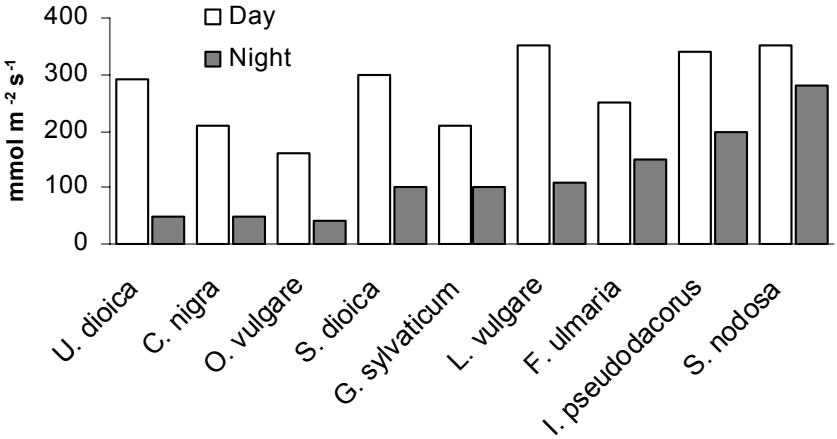
Given the fact that many upland species exhibit significant stomatal conductance (and hence ozone uptake) at night (and/or at low light intensities) this raises the question of whether night-time ozone exposure maybe deceptively damaging. To investigate this question, Caltha palustris was raised in controlled environment chambers at the University of Newcastle and exposed in duplicate chambers to charcoal/Purafil-filtered air or equivalent day-time and night-time ozone flux, achieved via the daily manipulation of atmospheric ozone concentrations in the night-time treatment based on measurements of stomatal conductance. Ozone flux calculations took account of differences in O_3 solubility due to lower night-time temperatures during night-time versus day-time ozone exposure.

Ozone flux was calculated using equation 1:

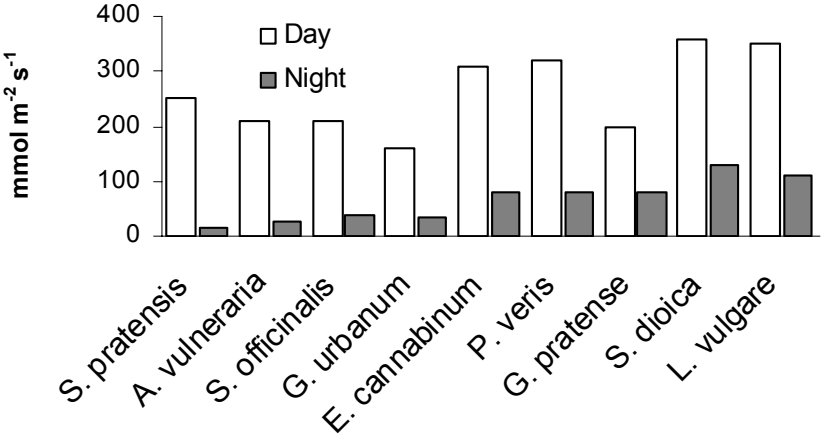
$F_{st} = c_{O3} * g_{H2O} * D_{H2O/O3} * s$ Equation 1

where, F_{st} represents ozone uptake per unit leaf area (nmol m^{-2} s^{-1}), c_{O3} is the hourly average ozone concentration (nmol m^{-3}), g_{H2O} is the stomatal conductance to water vapour (m s^{-1}), $D_{H2O/O3}$ represents the difference in diffusivity between ozone and water in air (0.613), and s represents the ozone solubility ratio coefficient in water (pH 7; mg l^{-1} per mg l^{-1} carrying gas)[5]

Screening of wild species: stomatal conductance in the field



Screening of wild species: stomatal conductance controlled environment chambers 1



Screening of wild species: stomatal conductance controlled environment chambers 2

[5] $\log_{10}s = -0.25 - 0.013T$ where, *s* represents the solubility ratio coefficient in water (pH 7; mg l^{-1} per mg l^{-1} carrying gas) and *T* is temperature (°C).

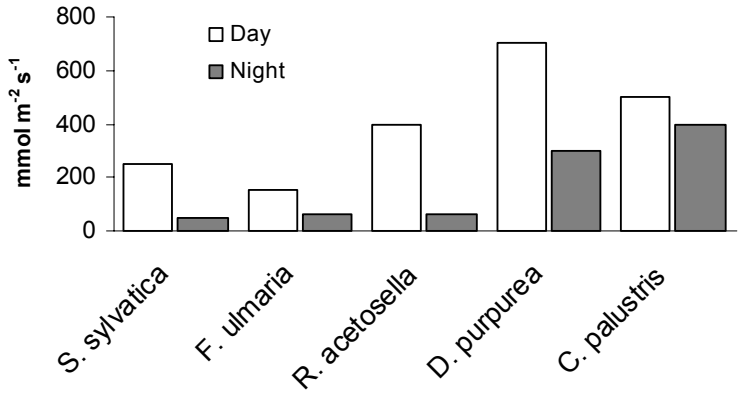


Figure 3.3 Day/night stomatal conductance measurements gathered in the field and on chamber-grown plants

Administration of 39 mmol O_3 m^{-2} at night versus day over a 7-week period resulted in a significant ($P< 0.01$) decline in the growth of *Caltha* under both regimes (Figure 3.4). However, the extent of the depression in growth was significantly greater for plants exposed during the night compared with the day (O_3 x day/night treatment $P< 0.01$). The entire experiment was repeated with similar conclusions. The findings lend clear support to the view that ozone uptake at night is particularly damaging (Winner *et al.*, 1989; Matyssek *et al.*, 1995; Tjoekler *et al.*, 1995). Upland species may be especially at risk because of the nature of the ozone climate at higher elevations. The findings indicate that there may be a need to 'weight' ozone uptake at night when considering exposure-response relationships for upland vegetation.

Night-time ozone uptake is believed particularly damaging because under these conditions cellular detoxification potential is considered to be minimal (since detoxification is believed to be driven by photosynthetically-derived reductant and/or immediate photosynthetic products e.g. ascorbic acid). In a bid to examine the mechanisms underlying the observations, ascorbic acid levels and the in vitro activity of key antioxidant enzymes were measured using techniques developed by the Newcastle laboratory (see Lyons *et al*., 1999)

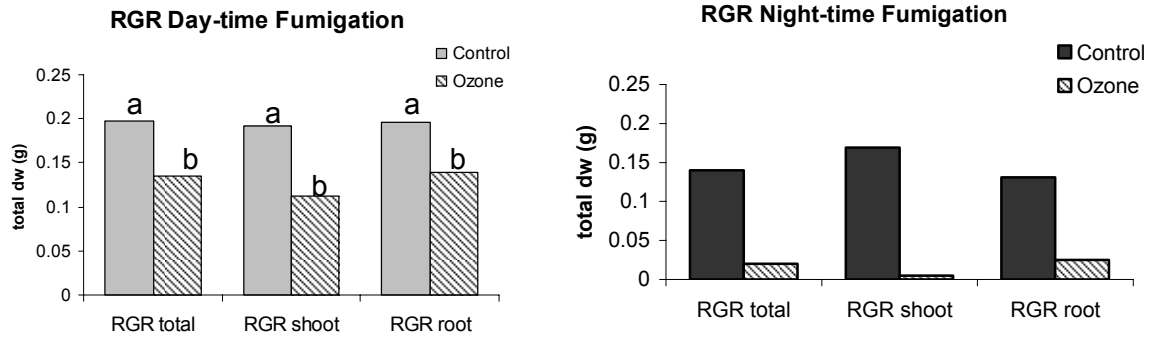


Figure 3.4. Relative impact of day versus night-time uptake of ozone. *Caltha palustris* was exposed in duplicate controlled environment chambers to either charcoal/Purafil-filtered air (controls) or ozone concentrations yielding a calculated ozone flux of 39 mmol O_3 m^{-2} (ozone) delivered over a 7-week period during the day-time or at night-time.

Biochemical investigations surprisingly indicated no significant diurnal changes in the cell wall-localised pool of ascorbic acid (Figure 3.5). These findings are consistent with other data recorded for wild species (Pignocchi, Foyer & Barnes, 2003; Bass & Barnes, 2005), but are strangely at odds with findings for crops (Smith, Foyer and Barnes, *in press*; Moldau, 1989). The reason for the discrepancy between wild species and crops is not yet understood, but suggests that an overnight decline in cell wall-localised acorbate is not the reason for the enhanced sensitivity of *Caltha*, at least, to ozone at night.

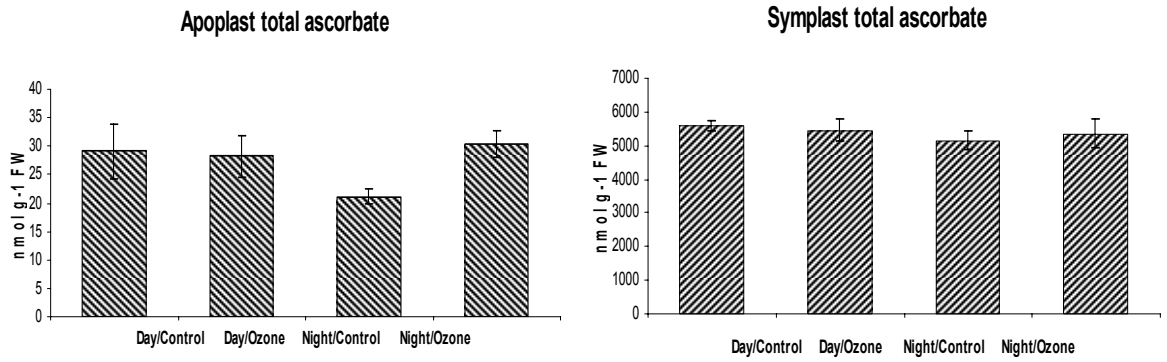


Figure 3.5. Day/night measurements of ascorbic acid content in apoplast/symplast. Measurements made on foliage of *Caltha palustris* during an experiment in which plants were exposed to equivalent day-time and night-time ozone flux (39 mmol m^{-2} over 7 weeks)

Moreover, in vitro measurements of key antioxidant enzyme activities suggested no evidence of an ozone x day/night interaction that would be consistent with the enhanced sensitivity of *Caltha* plants to ozone at night (Figure 3.6). However, it needs to born in mind that activity under these circumstances is measured under assay conditions providing optimal enzyme activity, and this situation maybe far removed from the situation at night-time in the plant, where reductant and/or substrates/co-factors could be limiting in vivo.

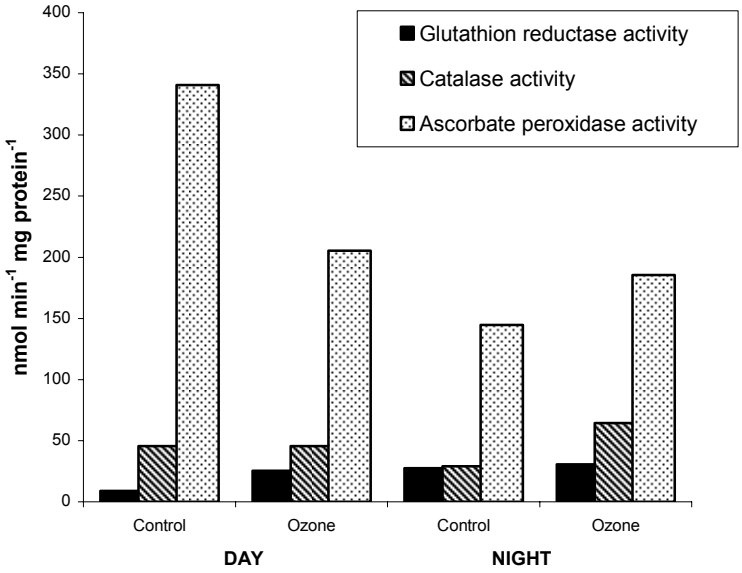


Figure 3.6 Day/night in vitro activity of glutathione reductase, catalase and ascorbate peroxidase in foliage of *Caltha palustris* exposed to equivalent day-time and night-time ozone flux (39 mmol m^{-2} O_3 over 7 weeks)

3.5.2.3 Is ozone more damaging at lower temperatures?

In upland situations, ozone levels are not only more stable during the day, but also over the course of the year. This situation gives rise to the potential for upland vegetation to be exposed during the early and later part of the year to elevated levels of ozone at lower temperatures than the norm in lowland situations. Since exposure to ozone at lower temperatures would increase the effective dose (due to increased ozone solubility and reduced capacity for ozone detoxification) there is the possibility the ozone exposure at lower temperatures is disproportionately detrimental (see Davison and Barnes, 1986). To examine this hypothesis, foxglove (*Digitalis purpurea*) plants were exposed to ozone in duplicate controlled environment chambers to charcoal-Purafil-filtered air or ozone at contrasting temperatures: 20-23^{o}C (ambient temperature) or 9-11^{o}C (low temperature). Ozone flux was manipulated in the low temperature regime so as to ensure equivalence (delivery of an effective dose of 40 mmol O_3 m^{-2} over 7 weeks) in both treatments (employing the principles adopted in Section 3.5.2.2). The exposure was sufficient to significantly ($P< 0.05$) reduce plant biomass in the low temperature regime, but not in the higher temperature regime, resulting in a O_3 x temperature interaction ($P< 0.01$). These data confirm the fact that O_3 uptake at low temperature maybe disproportionately damaging, and there may thus be a need to 'weight' seasonal exposures when considering the risks posed by ozone to upland vegetation

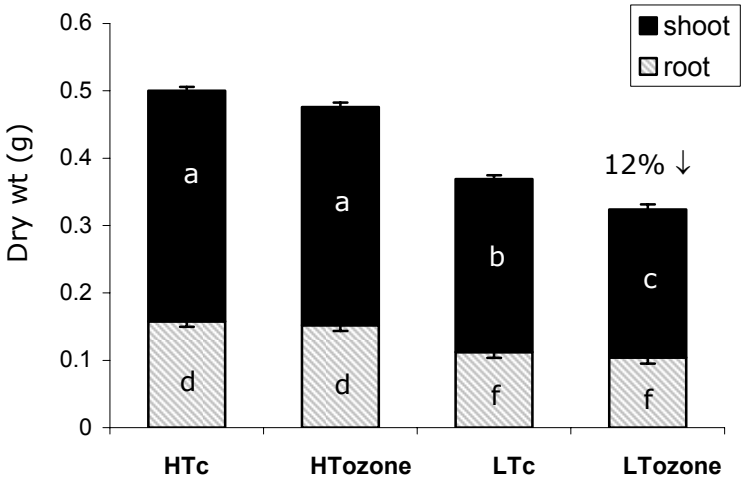


Figure 3.7. Impact of ozone at low (LT) versus high (HT) temperature. *Digitalis purpurea* was exposed to clean air or ozone in controlled environment chambers at 9-11°C or 20-23°C, respectively, with the atmospheric concentration adjusted in accordance with daily stomatal conductance measurements so as to deliver equivalent ozone flux (40 mmol O_3 m^{-2} over 7 weeks)

3.5.2.4 Is ozone more damaging when plants are parasitised (as they often are in the field) ?

The mesocosm experiments reported in Section 3.5.1 reveal the impacts of a conservative increase in ozone exposure in the uplands on a complex species-rich community, where one of the major factors driving species diversity is the influence of a hemi-parasite (hay-rattle) on the dominant grass species. Since there is some suggestion that hay-rattle maybe sensitive to ozone from studies conducted in controlled environment chambers (Ashmore, unpublished), we undertook a field-based study in open-top chambers to investigate the impacts of ozone on an O_3-sensitive (*Phleum pratense*) and an O_3-resistant grass (*Lolium perenne*) under *Rhinanthus* parasitisation. Individually-potted grass plants were raised in a cool glasshouse over winter, and pre-grown *Rhinanthus* seedlings introduced into half the pots in early spring. Of the large number of pots prepared, effective parasitism by *Rhinanthus* appeared in only *c.* 40% of cases. These pots were placed into triplicate open-top chambers receiving non-filtered air (NFA) and NFA plus 50 ppb ozone 8 h d^{-1} over the summer of 2005. No interactions were revealed between *Rhinanthus* parasitism and ozone impacts, but the variables resulted in an additive suppression of relative growth rate of the O_3-sensitve grass

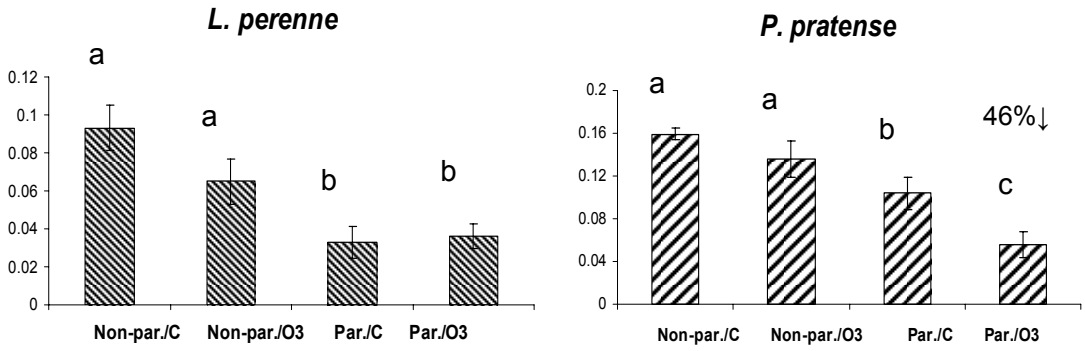
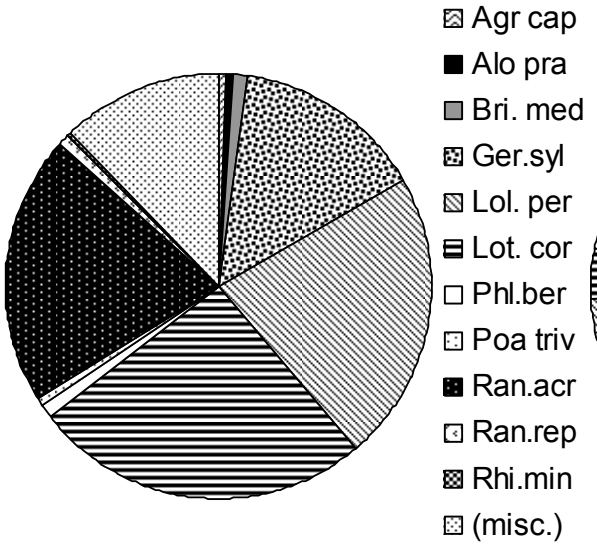


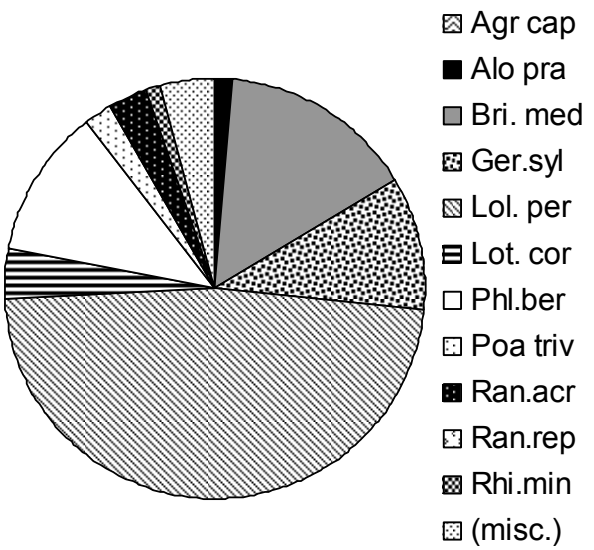
Figure 3.7. Impacts of *Rhinanthus* parasitisation on the response of an O_3-sensitive (*Phleum pratense*) and an O_3-resistant (*Lolium perenne*) grass to environmentally-relevant ozone exposure.

Appendix 1. OTC influence on mesocosm composition

NFA July 2004

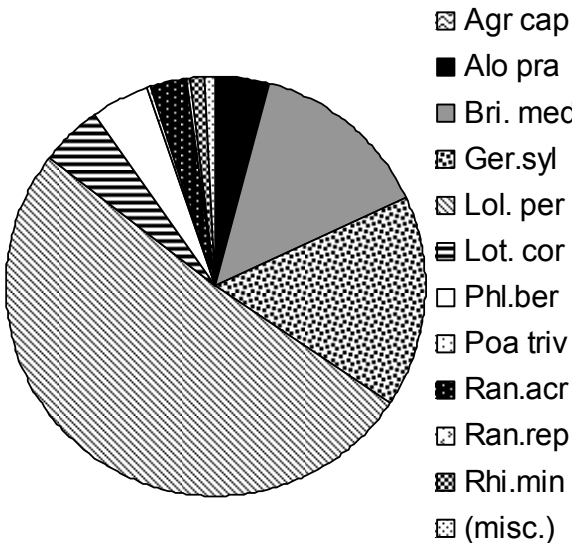


NFA July 2005



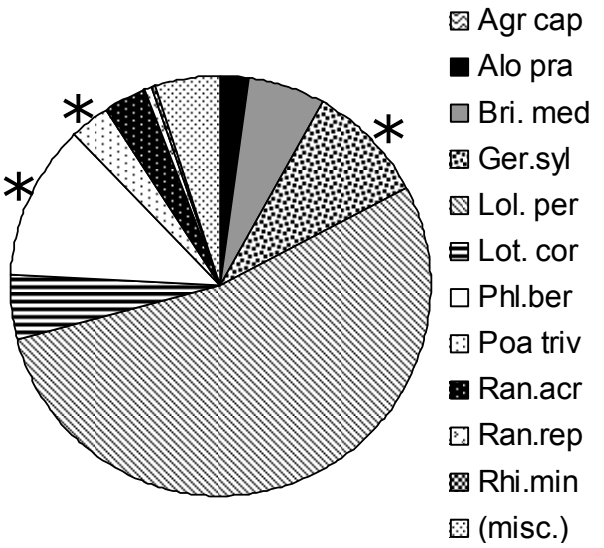
Appendix 2 Residual soil fertility

High Soil Fertility July 2005



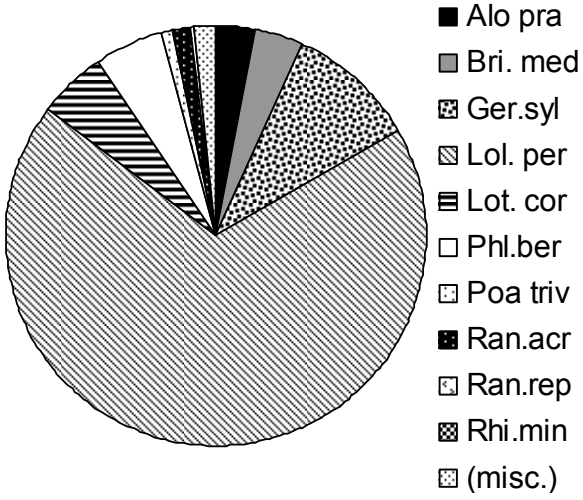
Low Soil Fertility July 2005

G. sylvaticum ↑ P.bertolonii ↓ P. trivialis↓



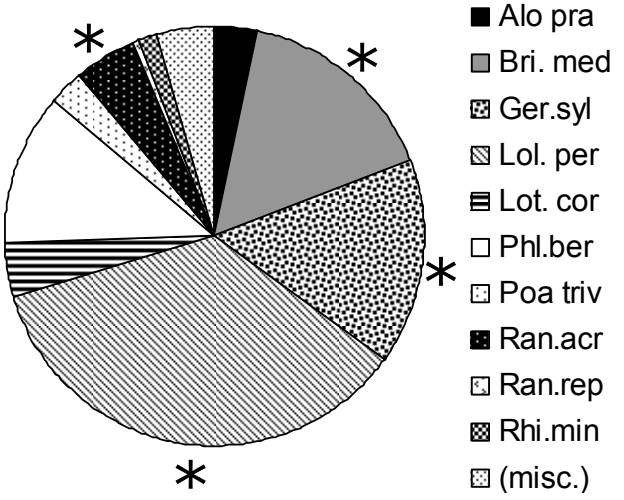
Appendix 3 Hemi-parasite treatment

R. minor absent July 2005



R. minor present (60 plants m^{-2}) July 2005

R. acris ↑ R. repens ↑ B. media ↑ L.perenne ↓



4 Interactive Effects of Oxidative Stress and Drought on UK Upland Vegetation

Lancaster University

4.1 Background

In recent years the mechanistic basis of plant response to tropospheric ozone has received some attention. Most recently, this has included observations of a marked effect of ozone on the calcium homeostasis of plants (McAinsh *et al*. 1996; Clayton *et al*. 1999). This has the potential to affect stomatal function by disrupting guard cell calcium-based signal transduction. Recent work by other groups has suggested that reactive oxygen species mediate abscisic acid signalling in guard cells (Pei *et al*. 2000). Reductions in stomatal aperture (or stomatal conductance, g_s) are frequently observed in response to ozone (Wellburn, 1994) and these two mechanisms provide possible explanations of this response. The hormones ABA and ethylene, both produced in increased quantities in droughted plants, can also be potent growth inhibitors in leaves and we can suggest that ozone might also act to reduce plant growth through a hormone-mediated effect.

Ozone induced reductions in stomatal conductance will affect plant water balance, growth and gas exchange. We also expect that limitations in stomatal conductance will reduce the flux of ozone into leaves and therefore the dose received by the leaf in a given exposure period. Plants that are well supplied with water are generally more sensitive to ozone exposure (Mansfield, 1998) and it is of interest to know whether this is a function of an effect of stomatal conductance on dose or due to a fundamental difference in metabolic sensitivity. Interactions between abiotic stress signalling pathways (including ABA and ethylene) and oxidative stress (Pastori and Foyer, 2002) suggest that there may be other important interactions between ozone exposure and plant water relationships. Predicted changes in UK rainfall patterns under climate change mean that interactions of this kind may have important effects on vegetation structure and functioning

The Lancaster work was designed to further elucidate the basis of genotypic variability in the responses of plants in the UK uplands to ozone exposure with emphasis on the effects of ozone exposure and drought treatments given in combination.

4.2 Experimental system

Pairs of species commonly found in natural or semi-natural British upland grassland communities were chosen to represent potentially contrasting sensitivities to elevated tropospheric ozone (Table 4.1). There were two grasses (*Phleum pratense* [timothy] & *Lolium perenne* [perennial rye grass]) and two legumes (*Trifolium repens* [white clover] & *Lotus corniculatus* [birdsfoot trefoil]). Plants were grown in a greenhouse containing growth chambers specially adapted for ozone fumigation (4 chambers each at control and

elevated concentrations of ozone). Temperature was regulated at 20ºC, while artificial lighting maintained a 14-hour photoperiod with a minimum PAR at canopy level of 200 µmol m^{-2} s^{-1}. The air supply to the growth chambers provided approximately one complete air change per minute, allowing ozone concentrations to be controlled by regulating the amount of pure ozone added to the incoming air stream (see below).

Table 4.1. Choice of species was based on apparent contrasts in sensitivity to ozone exposure taken from the literature review by Fuhrer et al. (2003). SI = Sensitivity Index, based on relative biomass at AOT40s of 15 and 3 ppm.h.

Species	SI
Trifolium repens	0.87 (sensitive?)
Lotus corniculatus	0.96 (tolerant?)
Phleum pratense	0.64 (sensitive?)
Lolium perenne	1.04 (tolerant?)

4.2.1 Elevated ozone treatments

Ozone fumigation of plants always began approximately 8 weeks after germination, and was continued for up to 8-9 weeks. In the first fumigation experiment, the control plants were exposed to fluctuations in the ambient ozone concentration, while the elevated ozone treatment was a constant 40 ppb above ambient. Thereafter, background ambient ozone was excluded almost entirely from the growth chambers, while the elevated ozone chambers were fumigated with air at around 70-80 ppb ozone. The resulting AOT40 values for the elevated ozone treatments were very similar after 2, 5 and 8 weeks of fumigation (Table 4.2). In both cases, the elevated ozone treatments were continued for 24 hours a day, in order to mimic the less marked diurnal fluctuations in ozone concentration typical of upland areas, which experience considerably higher night-time levels compared to lower altitudes.

4.2.2 Drought treatments and gas exchange measurements

Instantaneous measurements of gas exchange (net photosynthetic rate and stomatal conductance) were taken at growth light, temperature, humidity and CO_2 concentration. Maximum photosynthetic rate (an indicator of changes total photosynthetic capacity which might represent direct damage by elevated ozone) was also measured following at least 5 minutes' exposure to saturating light (1000 µmol m^{-2} s^{-1}) and CO_2 concentration (~2000 ppm). All measurements were taken using a portable IRGA (CIRAS-2, PP-Systems) at various times during and prior to, ozone fumigation. All plants were well watered prior to gas exchange measurements. In addition, the interactive effects of elevated ozone and soil drying on the above gas exchange parameters were investigated

by withholding water for periods of 24-48 hours (experiments 1 & 3) or by maintaining a constant water deficit over a number of days (experiment 2).

Table 4.2. Average AOT40 values (ppm.h.) for ozone exposure over three separate experiments after 2, 5 or 8 weeks.

Experi-ment	Weeks of O_3 exposure	Trifolium *repens*		*Lotus corniculatus*		*Phleum pratense*		*Lolium perenne*	
		Control	$+O_3$	Control	$+O_3$	Control	$+O_3$	Control	$+O_3$
1	2	0.35	9.81	0.35	10.39	0.35	9.23	0.30	8.65
	5	1.77	22.35	1.77	22.93	1.77	21.77	1.72	21.20
	8	-	-	3.01	33.30	3.01	32.15	2.96	31.57
2	2	0	8.22	0	8.77	0	7.68	0	7.13
	5	0	21.93	0	21.62	0	22.24	0	22.55
3	8	-	-	-	-	0	32.21	0	32.21

4.3 Results

4.3.1 Effects of elevated ozone on photosynthesis and stomatal conductance

After 5 weeks of exposure to elevated ozone, when AOT40 values had reached approximately 20 ppm.h (Table 4.2) net photosynthetic rate was unaffected in both grasses and in *Lotus* (Figure 4.1). However, in *Trifolium*, net photosynthetic rate was significantly lower in elevated ozone than in ambient air (Figure 4.1). After 8 weeks of exposure to elevated ozone (AOT40 of approximately 30 ppm.h, Table 4.2), *Phleum* too, showed a significant reduction in net photosynthetic rate (Figure 4.1). However, there was still no effect at all on the photosynthetic rates of *Lotus* or *Lolium*. This confirms that within the two species groupings, in terms of potential carbon gain and growth, *Trifolium* may indeed be more sensitive to elevated ozone than *Lotus*; and that *Phleum* may be more sensitive than *Lolium*. (N.B. data for *Trifolium* is unavailable for week 8 due to aphid damage.)

There were no significant reductions in maximum photosynthetic rate (A_{max}) in any species (Figure 4.2), indicating that elevated ozone probably caused no direct damage in terms of loss of photosynthetic capacity. The only possible exception is an apparent decline in A_{max} of *Lotus* over time, which was seen in the plants grown in elevated, but not ambient ozone levels (Figure 4.2). However, reductions in net photosynthetic rate appear to have been driven mainly by patterns of stomatal conductance in response to elevated ozone exposure (Figure 4.3). Reduced stomatal conductance may be an important mechanism to protect against oxidative stress by reducing the actual uptake of ozone by the leaves; however, it will also restrict CO_2 uptake, and hence limit the net photosynthetic rate. Hence, after 5 and 8 weeks respectively, both *Trifolium* and *Phleum* had lower stomatal conductance at elevated compared to ambient ozone levels (Figure 4.3), which corresponded to the reductions in photosynthetic rate (Figure 4.1).

A second fumigation experiment also found no significant effects of elevated ozone on photosynthetic capacity in any of the four species. In *Trifolium,* stomatal conductance was significantly reduced by elevated ozone (P = 0.030) even after 2 weeks of exposure (AOT40 ~8 ppm.h, Table 4.2); this was again associated with a decrease (though non-significant) in net photosynthetic rate (data not shown). No other species showed any significant effects of elevated ozone on photosynthesis or stomatal conductance at this level of exposure, confirming that *Trifolium* may be significantly more sensitive than other species, in terms of loss of productivity - at least when water supply is adequate.

4.3.2 Combined effects of elevated ozone and soil drying

At ambient ozone levels, *Lotus*, *Phleum* and *Lolium* all showed a marked decline in stomatal conductance in response to 24-48 hours of soil drying (Figure 4.3). In *Lotus*, this decline was mirrored in the elevated ozone treatment; however, both grass species showed a significantly weaker stomatal response to drought at elevated ozone levels than at ambient (Figure 4.3). Indeed, in the case of *Phleum*, there was no reduction in stomatal conductance at all. As a result, the net photosynthetic rate of *Phleum* and *Lolium* remained significantly higher at elevated ozone than at ambient throughout the period of soil drying (Figure 4.1). In contrast, the net photosynthetic rate of Lotus was markedly reduced by elevated ozone throughout the drought period (Figure 4.1). Such contrasting responses could have implications for interspecific competition within mixed grassland communities.

On the one hand, reducing stomatal conductance in response to elevated ozone and/or drought will help to conserve soil moisture, reducing the likelihood that plants will experience damaging water deficits. It will also reduce the actual uptake of ozone by the leaves, and therefore the risk of oxidative damage as a direct result of elevated background levels of ozone. On the other hand, net photosynthetic gain will be reduced, at least for the duration of the period of drought and/or exposure to high ozone concentrations. In contrast, the failure to reduce stomatal conductance in response to drought when exposed to elevated ozone (which was particularly noticeable in *Phleum*) will allow the plants to maintain a higher rate of photosynthesis – at least in the short term - while other species may be experiencing marked reductions in photosynthetic rate, perhaps even ceasing growth altogether. However, in the long term, maintaining a high stomatal conductance during drought will increase the likelihood that the plants will experience damaging levels of soil water deficit, combined with an increased risk of oxidative stress through higher levels of ozone uptake. Thus, the response of *Phleum* to combined oxidative and water stress may in the long run leave it more susceptible to damage from both.

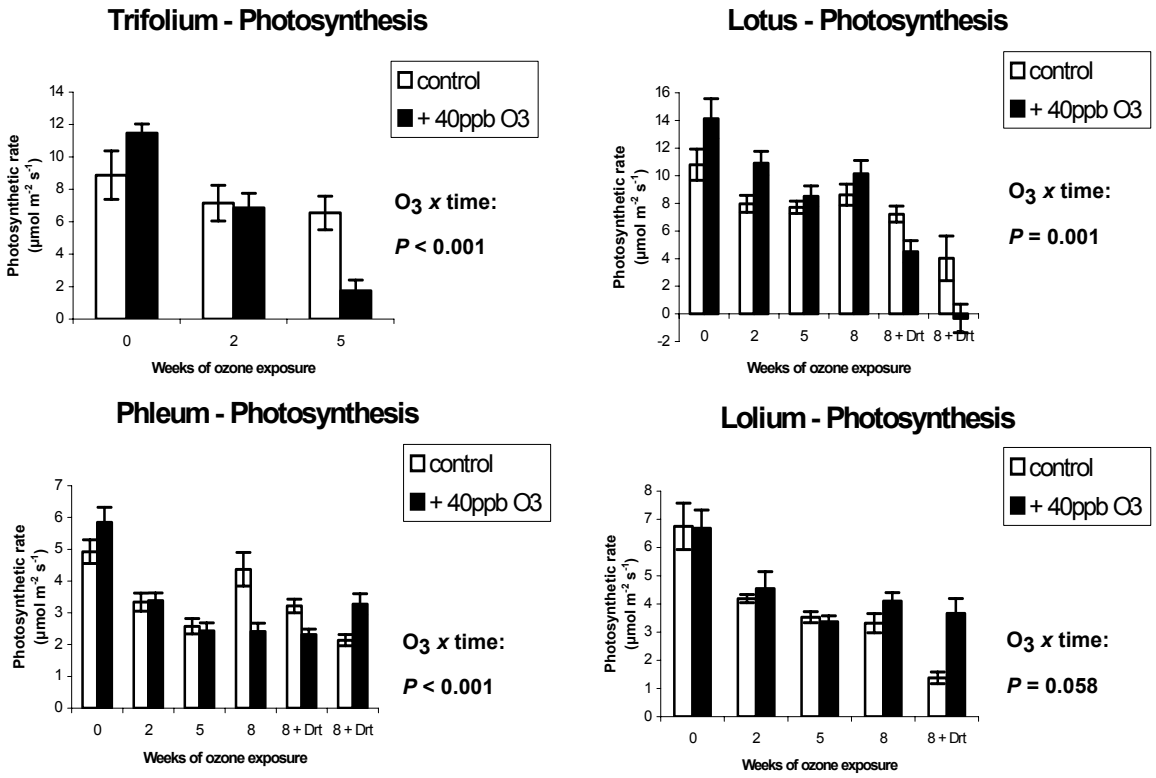


Figure 4.1 Net photosynthetic rate of *Trifolium*, *Lotus*, *Phleum* and *Lolium* after 2, 5 and 8 weeks of exposure to elevated ozone or ambient air control. At 8 weeks, measurements were repeated on droughted plants (Drt) after withholding water for 24 hours (and in the case of *Lotus* and *Phleum*, also after 48 hours without watering).

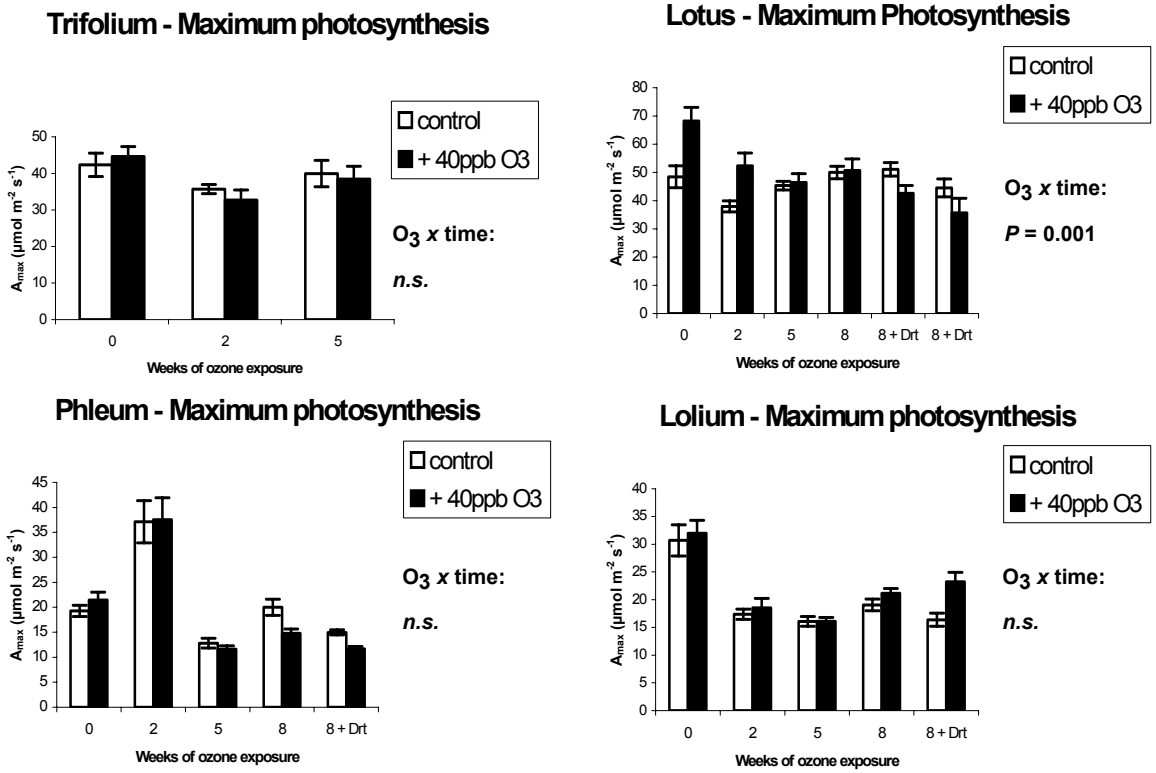


Figure 4.2. Maximum photosynthetic rate (A_{max}) of *Trifolium*, *Lotus*, *Phleum* and *Lolium* after 2, 5 and 8 weeks of exposure to elevated ozone or ambient air control. At 8 weeks, measurements were repeated on droughted plants (Drt) after withholding water for 24 hours (and in the case of *Lotus* and *Phleum*, also after 48 hours without watering).

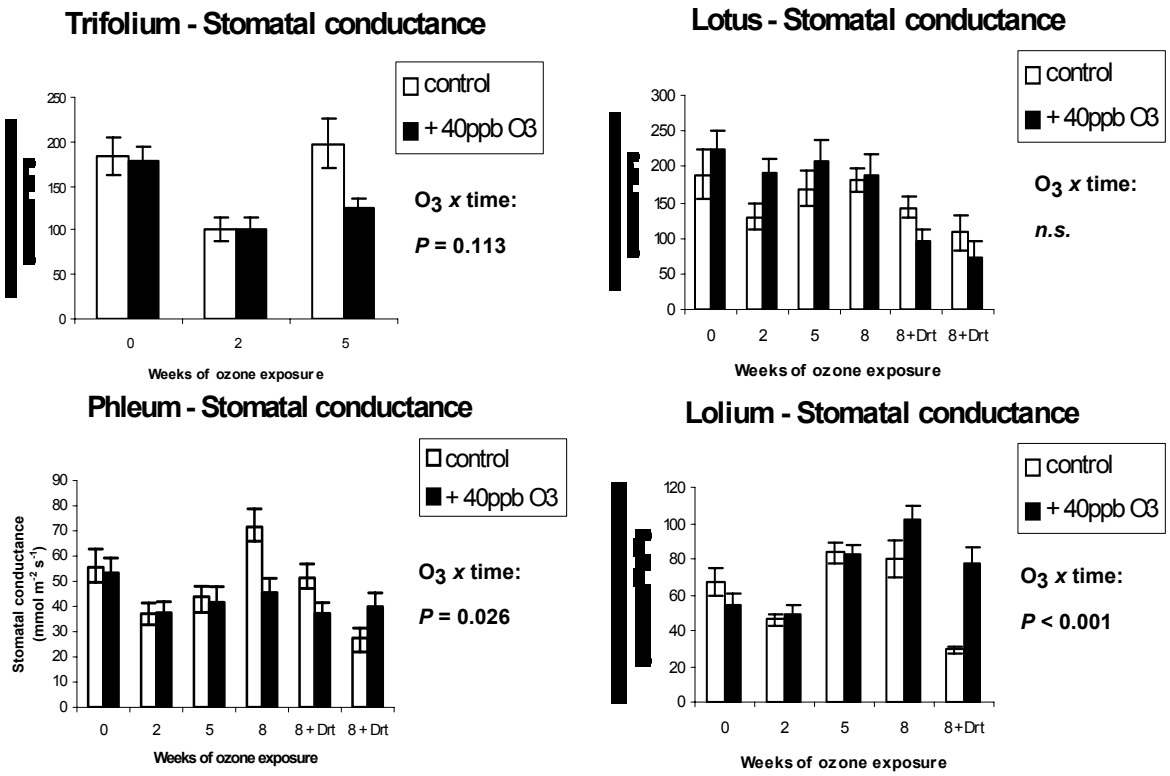


Figure 4.3. Stomatal conductance of *Trifolium*, *Lotus*, *Phleum* and *Lolium* after 2, 5 and 8 weeks of exposure to elevated ozone or ambient air control. At 8 weeks, measurements were repeated on droughted plants (Drt) after withholding water for 24 hours (and in the case of *Lotus* and *Phleum*, also after 48 hours without watering).

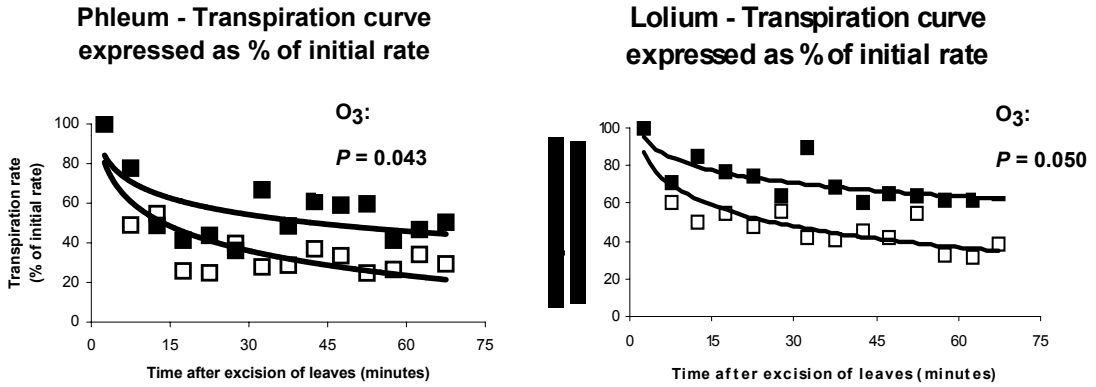


Figure 4.4. Curves showing the decline in transpiration rate of *Phleum* and *Lolium* leaves following excision from well-watered plants. The decline in transpiration rate represents the stomatal response to decreasing leaf water potential, independent from potential root-shoot signalling associated with drying soil. □ control; ■ elevated ozone.

Indeed, during the first 24 hours of droughting, total water loss from the soil was significantly higher in the elevated ozone treatment for both *Phleum* and *Lolium* (P = 0.027). Hence, at elevated ozone, both grasses were showing a smaller stomatal response to a greater soil moisture deficit. It would therefore appear that elevated ozone must somehow have been interfering with the signalling pathway leading from the detection of soil drying by the roots to the eventual stomatal response in the leaves. In

an attempt to understand at what point in this signalling pathway ozone could be taking effect, two further experiments were carried out on the same plants following re-watering and recovery from the drought treatment.

4.3.3 Stomatal response to decreased leaf water potential

To test the response to water deficit independently of root signalling, leaves of *Phleum* and *Lolium* were excised from the plants and the rate of water loss followed by measuring weight loss over 75 minutes. Values were converted to transpiration rates on a leaf area basis, and thereby represent the stomatal response to decreasing leaf water potential over a 75-minute period. The decline in transpiration rates following the excision of leaves is shown in Figure 4.4, plotted as % change in initial transpiration rates. In both Phleum and Lolium, the decline in transpiration rate – and by inference, the stomatal response to decreasing leaf water potential – was significantly less at elevated ozone levels than at ambient. This shows that the effect of ozone on the stomatal response to soil drying probably occurs within the leaf, downstream of any signalling from roots exposed to drying soil.

4.3.4 Testing the speed and magnitude of stomatal closure in response to darkness

To eliminate the possibility that the effects described above were not simply due to direct physical damage affecting the mechanics of stomatal control over water loss (either through a decrease in the absolute degree of stomatal closure possible, or the speed at which it can occur in response to environmental signals such as drought), the stomatal response to darkness was measured in *Phleum* and *Lolium*. Stomatal conductance was measured under growth light conditions and following 5, 10 and 15 minutes of darkness. Both species showed a marked and rapid decline in stomatal conductance in response to darkness, which was completely unaffected by ozone concentration (Figure 4.5). This result indicates that physical damage was unlikely to be the cause of the reduced stomatal sensitivity to soil drying, suggesting that elevated ozone concentration must have been affecting the signalling pathway within the leaf that would normally lead to a strong degree of stomatal closure.

4.3.5 Effects of constant level of soil water deficit

Th4.e stomatal responses to soil drying described above represent a dynamic response to a rapidly drying soil, rather than an end response to a longer-term, more stable level of soil water deficit. By growing plants in a greater soil volume, it was possible to maintain a fairly constant level of soil water deficit over a number of days (more than four, before any measurements were taken) by adding the appropriate volume of water to each pot daily. All species were kept at the same level of soil moisture deficit, which resulted in a similar degree of stomatal closure as seen after 24-48 hours of withholding water in the

first experiment. After 5 weeks of exposure to elevated ozone (AOT40 ~20 ppm.h, Table 4.2), and for >4 days, to soil moisture deficit, the stomatal conductance of Lotus and Lolium was unaffected by ozone concentration (data not shown). *Trifolium* continued to maintain a lower stomatal conductance at elevated ozone during drought (P = 0.030, data not shown) as it had done during previous periods with adequate water supply (see above). In contrast, during drought, the stomatal conductance of *Phleum* was significantly higher under elevated ozone than under ambient (Figure 4.6). This suggests that in *Phleum* exposed to elevated ozone concentrations, as well as the speed of the stomatal response to rapid soil drying being reduced, so is the overall degree of stomatal closure that can be expected over a more prolonged period of soil moisture deficit. This could have more serious implications for plant water use efficiency over timescales likely to influence seasonal patterns of soil drying, and the likelihood that plants will experience a damaging degree of drought. It also has the potential to significantly increase the total ozone uptake by leaves over the course of the growing season.

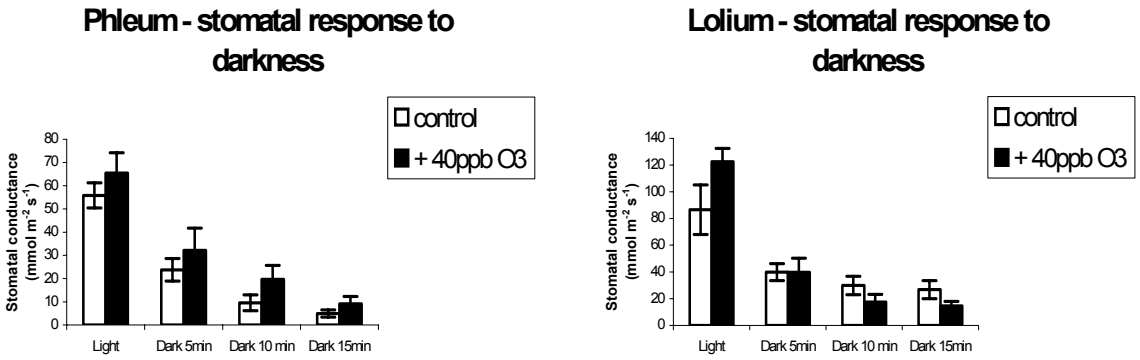


Figure 4.5. Stomatal closure of *Phleum* and *Lolium* in response to a step change from growth light conditions to complete darkness. There was rapid stomatal closure over a 15 minute period of darkness, but there were no significant effects of exposure to elevated ozone on the stomatal response of either species.

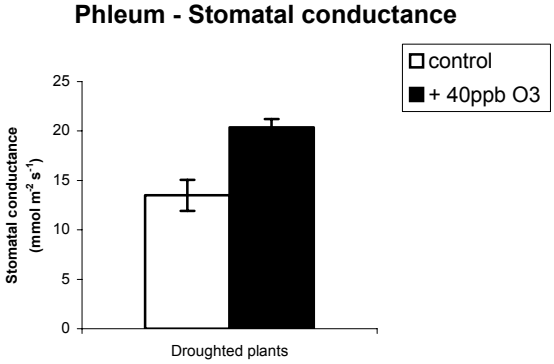


Figure 4.6. Stomatal conductance of *Phleum* after 4 days at a constant level of soil water deficit.

4.3.6 Further measurements of stomatal conductance (*Phleum* and *Lolium* only)

From the above, it seems clear that elevated ozone is reducing the stomatal response of *Phleum* to both a rapid soil drying and to a more stable level of soil moisture deficit. However, the situation in *Lolium* is less clear, with an apparently similar result for rapidly drying soil, but not for a stable soil moisture deficit. Further measurements taken after 8 weeks of exposure to elevated ozone (AOT40 ~30 ppm.h, Table 4.2) confirmed an effect of elevated ozone on the stomatal response of *Phleum* to soil drying, but unlike the first fumigation experiment, there was no such response in *Lolium*. Growth conditions were very similar in both experiments (including accumulated AOT40 values and the age of the plants at the time the measurements were taken). One possible explanation for the differing result is that in the first fumigation experiment, soil volume was smaller, resulting in a more rapid soil drying when water was withheld for 24-48 hours.

The stomatal response of *Phleum* to a 48-hour period of soil drying is shown in Figure 4.7, firstly as instantaneous measurements of stomatal conductance taken after 24 and 48 hours without water, and secondly as whole-plant average transpiration rates integrated over 0-24 and 24-48 hours. Transpiration rates were calculated from measurements of soil water loss (by weight) and whole-plant leaf areas. The stomatal conductance measurements show a similar, though not significant, effect to previous results for *Phleum*, with a reduced degree of stomatal closure in response to soil drying eventually resulting in higher stomatal conductance at elevated ozone concentrations. The lack of statistical significance may be partly explained by the fact that these measurements represent only a small number of individual leaves at one moment in time. In contrast, the calculated transpiration rates show the average rate of water loss (on a leaf area basis) for whole plants, time-integrated over a 24-hour period. In this case, the results are clear, with elevated ozone resulting in substantially and significantly higher transpiration rates over 0-24 and 24-48 hours of soil drying (Figure 4.7). This increase in transpiration rate was again associated with a significant increase in water loss from the soil ($P = 0.013$, data not shown), showing that the stomatal response to a given level of soil drying was substantially reduced under elevated ozone.

4.3.7 Bulk leaf abscisic acid (ABA) content of Phleum and Lolium

Bulk leaf ABA content was unaffected by ozone concentration in both species, both when well-watered and after 24 hours of soil drying (Figure 4.8). Hence, elevated ozone was not interfering with the stomatal response to drought by altering bulk leaf ABA levels through changes in de novo synthesis etc. This observation needs further investigation but does not rule out the possibility that oxidative stress has the potential to disrupt ABA-calcium guard cell signalling (rather than the delivery of ABA) (McAinsh *et al.* 2002) (see Figure 4.9).

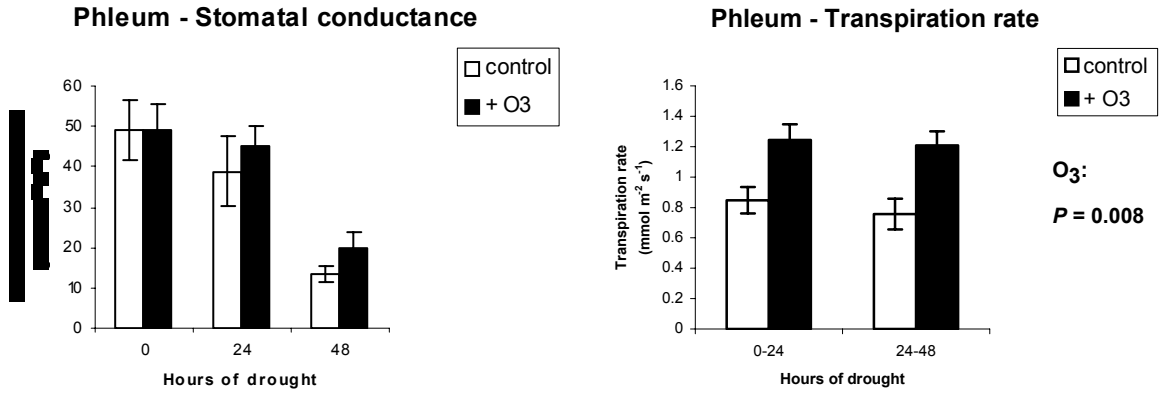


Figure 4.7**.** Stomatal conductance (instantaneous measurements on individual leaves) and transpiration rates (averaged for whole plants across periods of 24 hours) of *Phleum* during a 48-hour period of soil drying. There were no significant effects of ozone concentration.

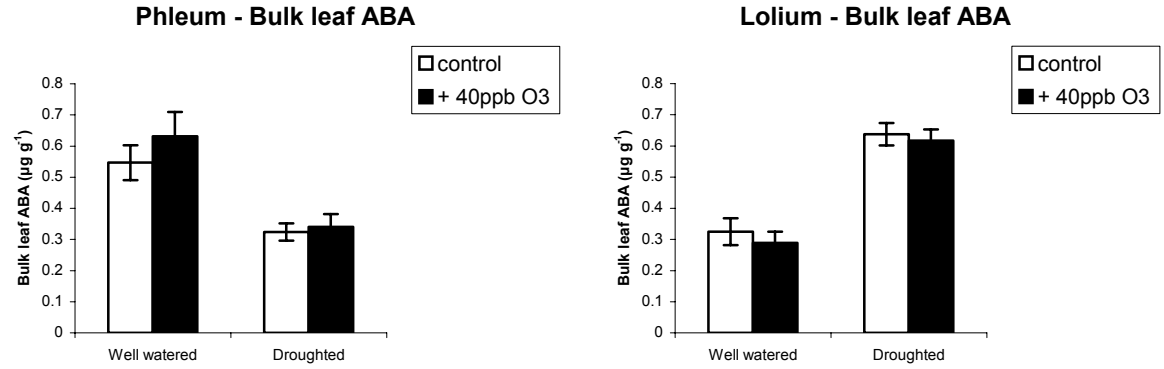


Figure 4.8. Bulk leaf ABA content of *Phleum* and *Lolium* after 8 weeks of exposure to ambient and elevated ozone, before and after 24 hours of droughting (leaves are the same as used for measurements in Figs. 1-3). There were no significant effects of ozone concentration.

4.4 Summary and conclusions

There may be no way of predicting a species' sensitivity to ozone through its family or functional type (Timonen, Huttunen & Manninen, 2004) or through known ecophysiological traits (Fuhrer *et al*., 2003), although some studies have identified trends (e.g. a generally high sensitivity of legumes, particularly *Trifolium* spp., compared to grasses: Gimeno *et al*., 2004). However, it is clear that there are major differences between species and/or across studies. Potential interspecific differences in plants' sensitivity to ozone stress are often assessed through measurements of relative growth rate or visible damage. Here, we have focused on physiological changes (in stomatal conductance and photosynthetic rate), which may occur independently of any significant reductions in growth rate but will potentially impact upon them. In this study, there was no evidence of oxidative damage causing any reductions in the photosynthetic capacity of the four species studied. Nevertheless, the more subtle physiological changes via modification of stomatal functioning and plant water relations may play an important role in patterns of interspecific competition (Figure 4.9).

Considering the legume species, Lotus appeared to be essentially unaffected by exposure to elevated ozone concentration, both in general and in terms of its physiological response to soil drought. In contrast, *Trifolium* consistently reduced its stomatal conductance above a certain cumulative level of exposure to ozone, both when well-watered and during periods of drought. While this stomatal response to some extent impacted on the net photosynthetic gain of *Trifolium*, it would also result in reduced uptake of ozone by the leaves, and increased water conservation during periods of drought. Thus, the risk of both oxidative and water stress may be substantially reduced over the course of a growing season. When well-watered, the same may apply to *Phleum*, but only after a higher cumulative ozone exposure.

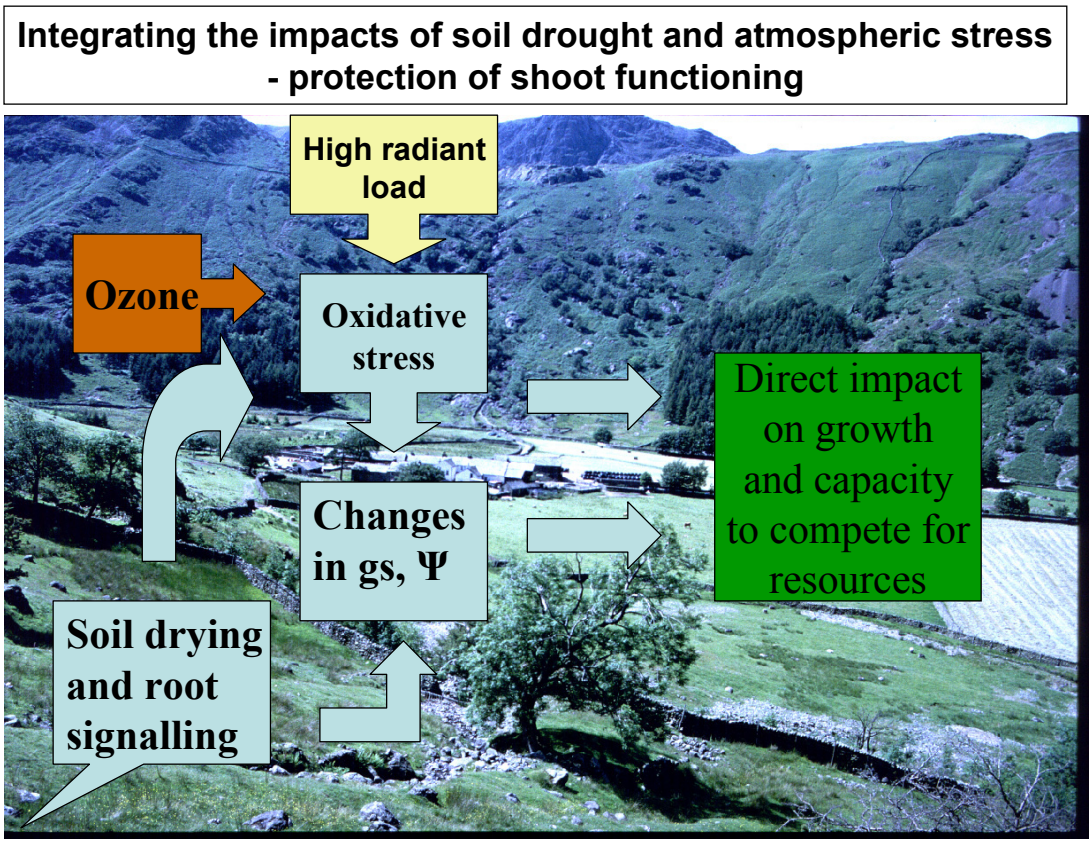


Figure 4.9. Schematic showing the proposed interaction between ozone exposure and soil drying on the development and functioning of upland vegetation

Of potentially greater significance is the response of *Phleum* to combined ozone and drought stress, because exposure to elevated ozone consistently reduced the strength of its stomatal response to soil drying (and to decreased leaf water potential independent of any below-above ground signalling). In the short term, this may allow the maintenance of a higher photosynthetic rate when compared to other species. However, during longer periods of drought, when exposed to high ozone levels, *Phleum* is likely to conserve less water, resulting in more rapid soil drying and an increased likelihood of exposure to damaging levels of water deficit. At the same time, while under increased susceptibility

to drought stress, the lack of stomatal closure will simultaneously increase its susceptibility to oxidative stress - the combination of which could be very damaging and would be expected to impact on both structure and functioning of the plant community. In the context of changing rainfall patterns under climate change, it is likely that responses of this kind will have important effects on vegetation structure and functioning. We have just begun to assess the interacting influence of ozone exposure and soil drying on the up-regulation of anti-oxidant systems. This topic will receive further attention in the up-coming project.

5 Field Measurements of the Ozone Flux to Vegetation

CEH Edinburgh: Mhairi Coyle, David Fowler, Eiko Nemitz, Gavin Philips, Robert Storeton-West, Rick Thomas

5.1 Summary

- Ozone and water-vapour flux have been measured over 4 different vegetation types for complete years at two sites (improved grassland and upland semi-natural moor/bog) and short summer campaigns at the other two (upland semi-natural grassland and oak forest). The measurements allow the split between stomatal and non-stomatal ozone deposition to be examined.
- Total ozone fluxes are greatest to the improved grassland and oak forest canopies due to their larger stomatal conductance. The fraction of deposition that was stomatal averaged 50 and 60% at each site during summer months, although overall it was only ~30% for the grassland.
- The fraction of ozone deposition that was stomatal for semi-natural moor/bog was ~20% on average with a median of 6%. During the summer month's stomatal uptake increased to ~60-90% of the total.
- When the canopy was dry non-stomatal ozone deposition was found to decrease with increasing surface temperature at three of the sites. The rate of decrease was similar at the two sites with most data: -9.8 s m^{-1} $^{o}C^{-1}$ for improved grassland and -7.2 s m^{-1} $^{o}C^{-1}$ for moor/bog (-44 s m^{-1} $^{o}C^{-1}$ for upland grassland). The most likely mechanism is the thermal decomposition of ozone mediated by compounds on or emitted by leaf surfaces.
- A similar response was found with solar radiation over dry surfaces (-0.27 s m^{-1} [Wm^{-2}]$^{-1}$ for improved grassland; -0.30 s m^{-1} s m^{-1} [Wm^{-2}]$^{-1}$ for moor/bog; -0.74 s m^{-1} [Wm^{-2}]$^{-1}$ for upland grassland) which is attributed to the correlation of surface temperature to radiation and possibly additional photolytic reactions occurring at the leaf surface.
- The median R_{ns} was lower for wet surfaces than dry at the two sites with data (204 s m^{-1} wet, 282 s m^{-1} dry for improved grassland and 248 s m^{-1} wet and 307 s m^{-1} dry for moor/bog) and decreased slightly with increasing temperature and solar radiation. This indicates that aqueous chemistry is most likely responsible and it may be enhanced by additional photolytic/photochemical reactions during the day-time.
- R_{ns} for dry surfaces increased with increasing relative humidity (*RH*) up to ~60-70% but decreased with *RH* above this. This would occur if a water film built up on the surface as *RH* increase, initially blocking the sites for thermal decomposition but then allowing aqueous reactions to occur as the film thickens.

- There was some dependence of R_{ns}(dry) on friction velocity (u_*) for the moor/bog but not the grassland, which is attributed to the different canopy structures. No significant effect of surface area index (*SAI*) was found at either the improved grassland or moor/bog although it was expected the R_{ns} would decrease as *SAI* increased. It is proposed that a similar surface area is exposed to the air as the canopy grows and it is only with a open structure, such as the moorland, that more air penetrates the canopy with increasing wind-speed or u_*.
- Further work is required to derive a model for the observed R_{ns} responses but some consistent patterns have been found between different sites and canopies.

5.2 Introduction

As ozone is highly reactive, it rapidly deposits to most surfaces and it is also removed by plants taking it in through their stomata. It is accepted that the stomatal uptake of ozone causes most damage to vegetation (Fuhrer *et al* 1997). The downward flux of ozone can be easily measured in the field using micrometeorological methods. However, distinguishing between different deposition pathways, eg stomata and leaf cuticle, is more difficult but necessary if the processes controlling deposition are to be fully understood and the threat to vegetation assessed.

The aim of this work package was to measure the flux of ozone to representative vegetation in the field and quantify the split between stomatal and non-stomatal uptake. Measurements were undertaken at 4 sites, two of which ran continuously and the other two for short campaign periods. The data from each site are summarised in this report and used to examine the amount of deposition that occurs via stomatal uptake and that to other plant surfaces (non-stomatal). At each site the canopy water-vapour flux was measured alongside the ozone flux which allows the stomatal uptake to be estimated (as described in the appendix). However, these measurements are restricted to periods when the vegetation is completely dry and so to expand the dataset models were also applied, which are also described in the appendix.

It is also possible to separate the stomatal and total non-stomatal pathways by considering only night-time measurements or measuring and modelling the stomatal flux. Previous work using both of these approaches has identified surface temperature, solar radiation, surface wetness and relative humidity as possible controlling mechanisms on the non-stomatal flux (Coe *et al.*, 1995; Fowler *et al.*, 2001; Rondon *et al.*, 1993; Zhang *et al.*, 2002).

Increasing surface temperature and solar radiation increase the ozone flux to dry surfaces. It has been hypothesised that this is due to the thermal decomposition of ozone or photolytic/photochemical destruction of ozone; mediated by compounds on, or emitted

by, leaf surfaces. The effect of surface wetness and humidity is less clearly defined as some studies found the ozone flux increased when the surface was wet while others that it decreased. In some circumstances surface water may block the pathway for ozone deposition as ozone has a low solubility in water and so the flux decreases. However, if the chemistry of the surface water allowed significant uptake of ozone by aqueous phase reactions then the flux may increase.

To better understand the factors controlling non-stomatal deposition the variation in non-stomatal canopy resistance with surface temperature, solar radiation and humidity is examined at each site.

5.3 Site Descriptions

5.3.1 Auchencorth Moss

The Auchencorth Moss field site just south of CEH, in the Borders region (Figure 5.1a), and has been monitoring total ozone deposition for 10 years, with periods of water-vapour flux measurements. The total ozone flux to the canopy is measured using a 3-point gradient system and in May 2002 continuous eddy-correlation measurement of water-vapour and carbon-dioxide fluxes was added to the site (Figure 5.1b). This allows the partitioning between stomatal and non-stomatal ozone fluxes to be studied. The pre-2002 measurements were reported in Fowler *et al* 2001 who showed that 60-79% of the total ozone flux was non-stomatal and that the non-stomatal surface resistance (R_{ns}) decreased with increasing solar radiation (St) and surface temperature ($T_{z0'}$). The more recent data are summarised here and the non-stomatal uptake is re-examined.

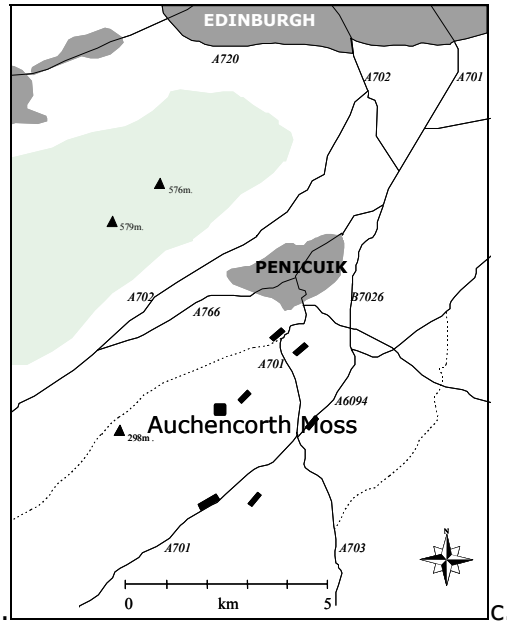
Table 5.1. Summary of measurements and instruments at Auchencorth Moss (Time period analysed: 27/06/2002 15:00 to 31/12/2004 24:00).

Instrument/System	Parameters measured	Started	%Data capture for time period (%QA/QC and filtering)
Gradient: UV-photometric ozone analyser	$\|O_3\|$ at 3 heights	January 1995	92% (60%)
Gill sonic anemometer	Wind speed and direction, turbulence, air temperature	January 1995	89% (75%)
LICOR 7000	CO_2, H_2O vapour fluxes	July 2002	83% (62%)
Meteorological mast	Total solar radiation, PAR, net radiation, air temperature, soil heat flux, soil temperature, surface wetness, rainfall	January 1995	73-98%

Other meteorological variables are measured using standard instrumentation, as outlined in Table 5.1. The vegetation at the site is typical of rough, upland moor or bog with large amounts of *Deschampsia flexuos, Eriophorum* varieties, various mosses and other wild species (Table 5.2, Figure 5.1c). The canopy height is estimated using a model derived from detailed measurements made in 1995. More recent studies of the canopy for the

Carbomont project included measurements of canopy biomass and LAI. These show that the LAI varies from 0.2 to 2 throughout the year but a seasonal model has yet to be derived from these data. The total surface area (*SAI* – surface area index) is of interest when studying R_{ns} and so *SAI* is assumed to follow the same cycle as canopy height with monthly values of 1, 1, 1, 1.2, 1.2, 1.5, 2, 2, 1.5, 1.2, 1, 1 (from Jan to Dec).

a.



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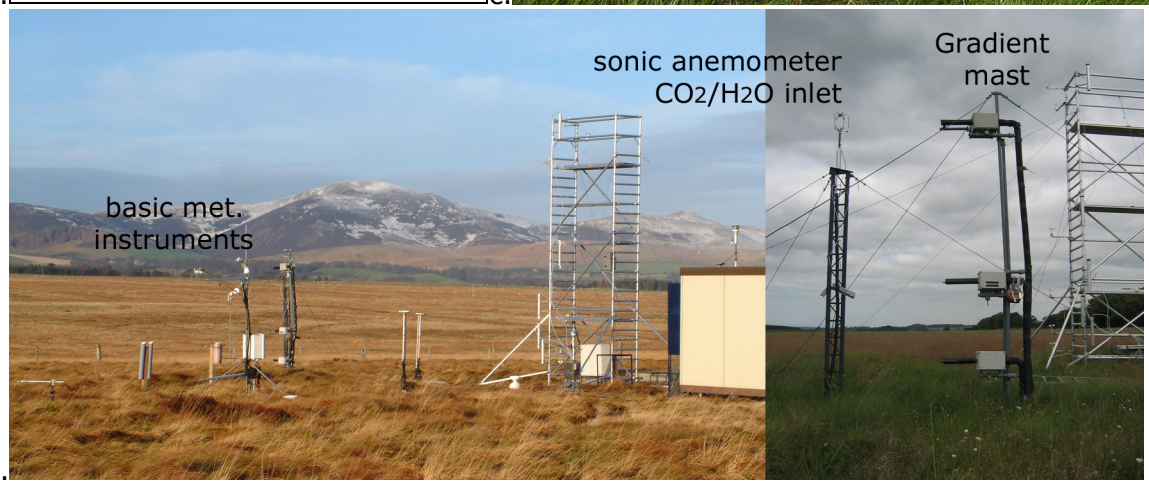


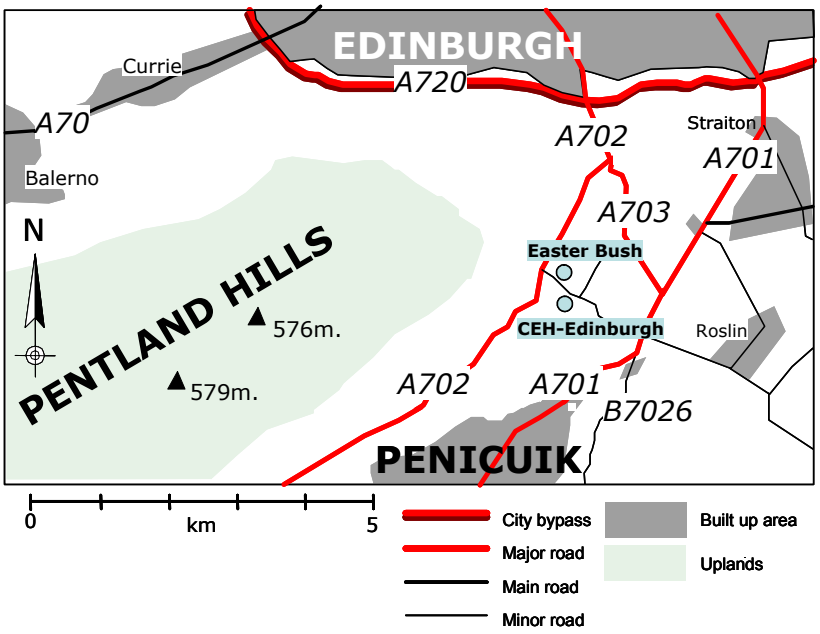
Figure 5.1 a. Location of the Auchencorth Moss field site (latitude 55° 47′ 30′′ N, longitude 3°14′ 20′′ W) b. Photographs of the site showing the relevant instrumentation c. Typical summer vegetation.

Table 5.2. Plant species found at Auchencorth, listed in descending order of dominance (Flechard, 1999)

Dominant vascular plants	Dominant bryophytes	Also present
Deschampsia flexuosa	*Polytrichum commune*	*Vaccinium myrtillus*
Molinia caerulea	*Polytrichum formosum*	*Erica tetralix*
Eriophorum vaginatum	*Brachytecium* spp.	*Potentilla erecta*
Eriophorum angustifolium	*Sphagnum tenellum*	*Galium oratum*
Festuca ovina	*Sphagnum papillosum*	*Carex ovalis*
Festuca rubra	*Sphagnum compactum*	*Carex nigra*
Agrostis stolonifera	*Sphagnum cupsidatum*	*Scirpus cespitosus*
Anthoxanthum odoratum	*Polytrichum urnigerum*	*Juncus effusus*
Nardus stricta	*Rhytidiadelphus squarrosus*	*Juncus squarrosus*
Trichophorum cespitosum	*Aulacomnium Palustre*	*Calluna vulgaris*

5.3.2 Easter Bush

This site is also located in southern Scotland (3”12’ W, 55” 52’ N, elevation 190 m above sea level), close to CEH-Edinburgh and consists of two grass fields of approximately five hectares each (Figure 5.2a). The equipment was placed on the boundary of the 2 fields which runs NW to SE (Figure 5.2b), allowing measurements in all wind directions (excluding the boundary). The canopy is predominately *Lolium perenne* (>90%, Figure 5.3a) and the fields were intermittently used for grazing and cut for silage in 2001 and 2002. The canopy height was regularly measured and leaf area index (LAI) estimated using a relationship derived from concurrent measurements of both parameters in 1999 and 2002 (Figure 5.3b).



b.

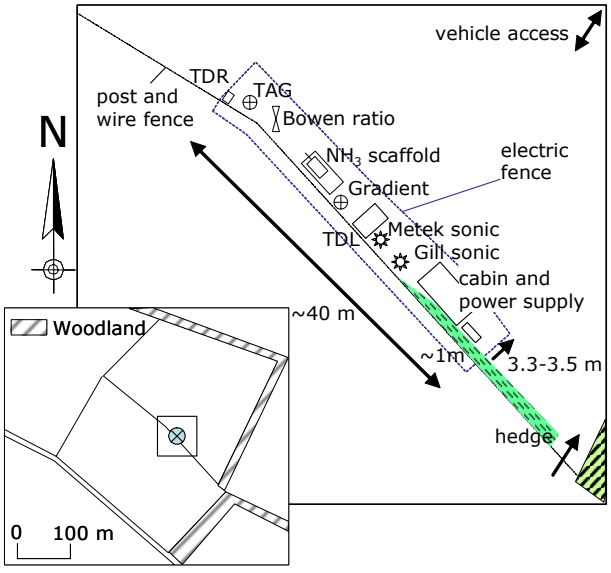


Figure 5.2. a. The location of Easter Bush and CEH Edinburgh. b. A sketch and photo of Easter Bush showing the location of the equipment enclosure.

Measurements with the following groups of instrumentation were made from May 2001 to May 2005: sonic anemometers and an open-path LICOR 7500 to measure wind direction, turbulence, sensible-heat flux, CO_2 and water-vapour flux; a 5–point gradient of ozone concentration (UV-photometric analysers), wind-speed (cup-anemometers) and temperature (fine-wire thermocouples) which give the ozone flux and turbulence; solar radiation, net radiation, soil heat flux, soil temperature, surface wetness (PCB grid), relative humidity, air temperature and soil moisture content (TDR – time domain reflectometry Campbell Scientific).

Data from the start of measurements until the end of 2004 were thoroughly reviewed and analysed using standard protocols, similar to those defined for the EUROFLUX and FLUXNET programs (Aubinet *et al.*, 2000; Fowler *et al.* 2001; Lee *et al.*, 2004). The resulting data set of aerodynamic resistance (R_a, s m^{-1}), bulk-canopy boundary layer resistance (R_b, s m^{-1}), sensible heat-flux (H, W m^{-2}), latent-heat flux (λE, W m^{-2}), water-vapour flux (E, g m^{-2} s^{-1}) and ozone flux (F_{O3}, ng m^{-2} s^{-1}) are used to examine the stomatal (R_{c1O3}, s m^{-1}) and non-stomatal surface resistance (R_{ns}, s m^{-1}) to ozone deposition. Table 5.3 summarises the instrumentation used and gives the overall data capture achieved.

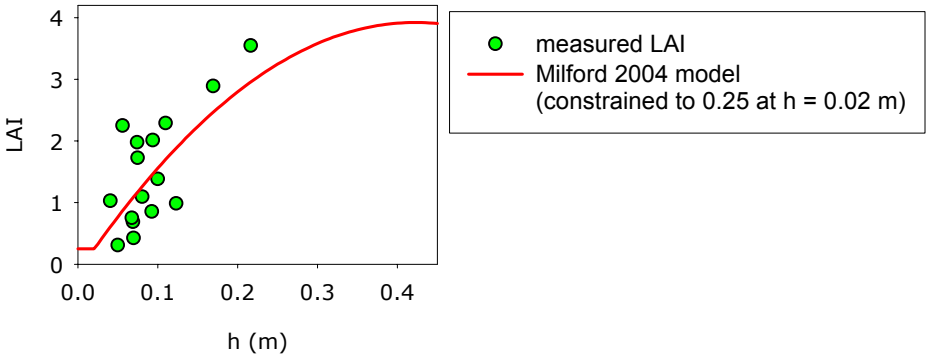
Table 5.3a Instrumentation employed at Easter Bush.

System Group	Components	Parameter and Label	Start Date	End Date
Bowen Ratio	2 Fine-wire type-E thermocouples	Air temperature gradient T_l, T_u	18/5/01	ongoing
	2 Soil Heat Flux plates	Soil Heat Flux (G) HF_{s1}, HF_{s2}	18/5/01	
	Averaging temperature probes	Soil temperature T_s	18/5/01	
	RH/T probe	Relative humidity and air temperature RH, T	3/4/03	
	Pyranometer	Total solar radiation, St	18/5/01	
	PAR sensor	Photosynthetically active radiation, PAR	3/4/03	
	2 Net radiation sensors	Net radiation, Rn_1, Rn_2	18/5/01	
	Surface Wetness PCB	Presence and duration of surface wetness SW	8/6/01	
	Tipping bucket	Rainfall	18/5/01	
Gradient	3 Fine-wire type-E thermocouples	Air temperature gradient T_1, T_3, T_5	22/5/01	ongoing
	5 cup-anemometers	Wind-speed gradient U_1 to U_5		
	ozone analyser	Average ozone concentration gradient, χ_{O3_1} to χ_{O3_5}		
Eddy-correlation	2 sonic anemometers	Turbulence parameters and wind direction u', w', v' etc and Wd.	15/5/01	ongoing
	LICOR CO_2/H_2O analysers	CO_2 and H_2O flux and concentration $\chi_{CO2'}$, χ_{CO2} $\chi_{H2O'}$, χ_{H2O}		ongoing
Other	TDR – time domain reflectometer	Soil water content at 4 locations in each field SWC_{SW1} to SWC_{SW4}, SWC_{NE1} to SWC_{NE4}	25/6/02	ongoing

Table 5.3b Summary of raw and final data capture (%) for the main components of the trace-gas flux measurements (26/5/01 to 31/12/04).

	u_*, H (Gradient, Gill, Metek, Combined)		O_3 (Gradient, Gill, Metek, Combined)		H_2O (dry daylight)		CO_2	
	Raw	Final	Raw	Final	Raw	Final	Raw	Final
2001	72, 96, ND	84	72, 95, ND	39	97	53 (43)	97	41
2002	62, 88, 64	76	61, 79, 56	55	88	74 (14)	88	14
2003	69, 89, 83	89	68, 80, 79	74	86	57 (29)	86	30
2004	65, 92, 85	79	53, 78, 76	65	93	64 (29)	92	28
Overall		78		69		26 (10)		26

a.



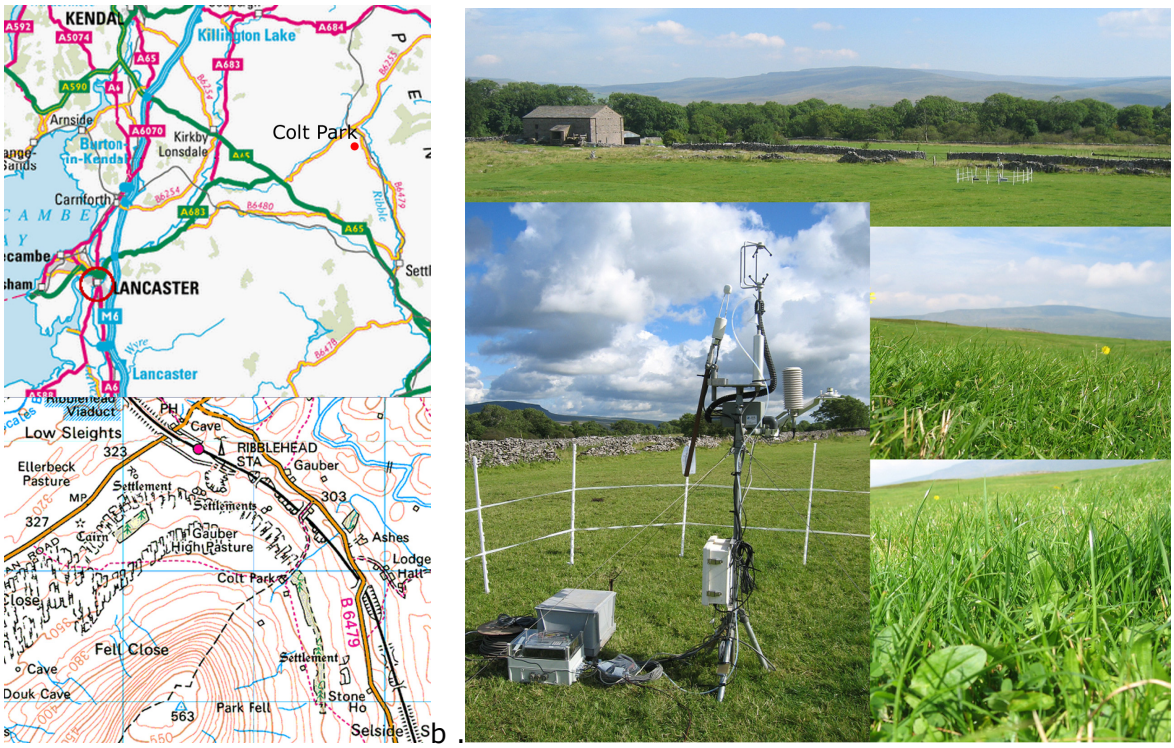
b. $LAI = (-22.75h^2)+(19.22h)-0.14$

Figure 5.3. The relationship between *h* and *LAI* for *lolium perenne* as derived by Milford, 2004 and measurements for 2002 to 2004.

5.3.3 Colt Park

From the 5th of August to the 5th of October 2005 the flux of O_3 and H_2O were measured, along with other meteorological variables, over an upland limestone grassland at the English Nature site, Colt Park (SD77140, -1.8°W 49.9°N) in the Ingleborough National Nature Reserve (Figure 5.4). The meadows are very rich in species and managed using hay harvesting and grazing to maintain their diversity. The flux measurements began a week after the meadow had been harvested and some grazing occurred during the measurement period. The canopy was therefore quite short throughout (3 to 15 cm, Figure 5.4b) and little growth occurred. Samples of plants from this site were also used by sensitivity screening work package within this project.

The ROFI (Rapid Ozone Flux Instrument) for measuring ozone flux by eddy-correlation was deployed at this site alongside an open-path LICOR7500 CO_2/H_2O sensor, Gill sonic anemometer and other basic meteorological instruments, as summarised in Table 5.4.



a. b .

Figure 5.4a. Location of the Colt Park site in the Yorkshire Dales National Park, near to the Ribblehead viaduct, b. photos of the instrumentation and vegetation.

Table 5.4 Instrumentation and data capture at Colt Park **(05/08/05 10:30 to 06/10/05 00:00)**

Instrumentation	Variables	%Datacapture (filtered)
ROFI & 2BTech UV O_3 analyser	O_3 Flux and concentration (F_{O3}, $\|O_3\|$)	99% (84%)
Sonic anemometer	Turbulence, wind speed and direction	92% (76%)
LICOR75000	CO_2/H_2O flux & concentrations (F_{CO2}, $\|CO_2\|$, F_{H2O}, λE $\|H_2O\|$, e)	98% (42%)
Pyramometer, net radiometer, soil heat flux, soil temperature, surface wetness, relative humidity, air temperature	Total solar radiation (St), Rn, T_{soil}, SW, RH, T_a	98% (92%)

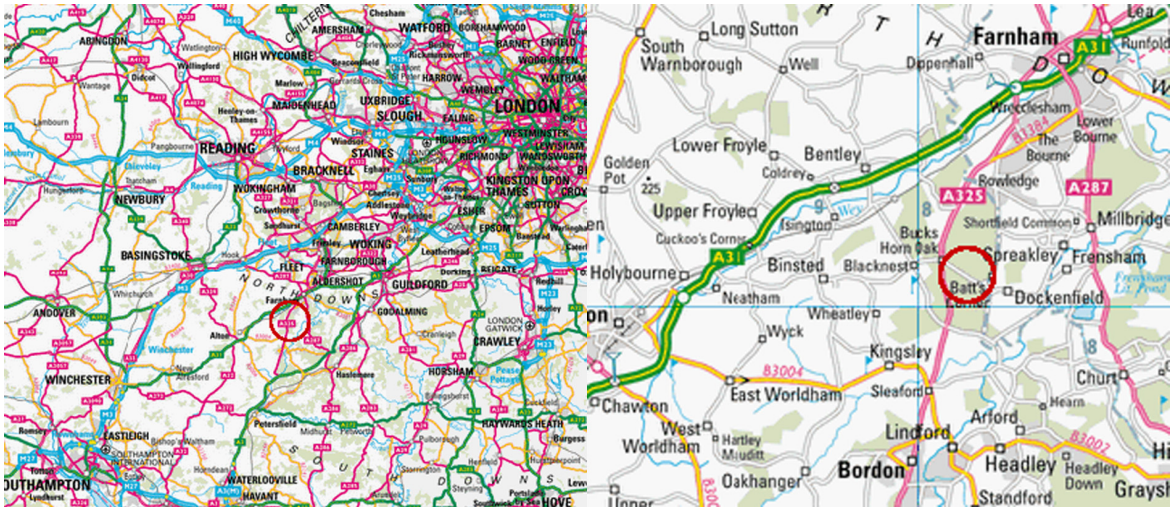
5.3.4 Alice Holt

Alice Holt is an oak forest (*Quercus robur* and *petraea*) near Farnham SW of London, managed by the Forestry Commission (51° 7' N; 0° 51' W, Figure 5.5). The measurements of ozone flux were made using a ROFI and Monitor Labs ozone analyser as part of the Defra funded Acid Deposition Processes project (Defra RMP 2258/CEH C02379) from the 16th of July to the 18th of August, 2005. The instruments were placed on the Forestry Commissions flux tower at a height of ~13 m which was 4 m above the canopy top. Although measurements of water-vapour flux are made at the site they were not available for this analysis and so a model of stomatal conductance was used, as supplied by Mark Broadmeadow (Forestry Commission) and described in the Appendix. The data used are summarised in Table 5.5.

Table 5.5 Instrumentation and data capture at Alice Holt (16/6/05 to 18/8/05).

Parameter	Methodology	%Data Capture	Measured By
Temperature, vapour pressure, PAR, humidity, soil moisture		100	Forest Research Commission
O_3 concentrations	Thermo 49C UV photometric detector	100	CEH Edinburgh & Forest Research
O_3 fluxes	ROFI Eddy Covariance	95 (73)	CEH Edinburgh
Windspeed, wind direction & turbulence	Gill Solent ultrasonic anemometer	95 (78)	CEH Edinburgh

a.



b.

c.

d.

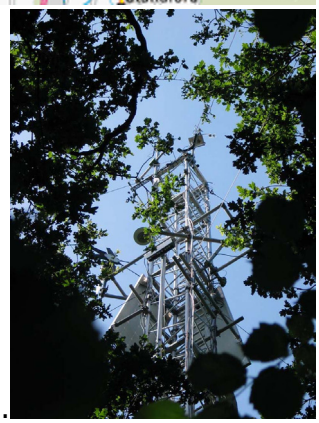


Figure 5.5 The Alice Holt field site, a. location, b. aerial view of the Straits enclosure (red dot indicates position of the measurement tower), c. view of the canopy from the top of the tower, d. the instrument tower.

5.4 Results

The measurements at each site were processed using standard filtering methodologies for removing poor quality data and to ensure the conditions required for micrometeorological methods apply, as outlined in the Appendix. The datasets are summarised in Table 5.6 and described in the following sections.

5.4.1 Ozone Concentrations and Total Fluxes

The results obtained from each site are comparable to measurements made over similar vegetation (Coe*, et al.*, 1995;De Miguel*, et al.*, 1999;Enders, 1992;Finkelstein*, et al.*, 2000;Fuentes*, et al.*, 1992;Gerosa*, et al.*, 2005), although there are currently few other examples for communities with the same characteristics as these. The frequency distribution of the total ozone flux is typically log-normally distributed while the ozone concentration is normal and, with the exception of concentrations at Alice Holt, this is the case at each site as shown in Figure 5.6. The ozone concentration is slightly skewed towards smaller values at Alice Holt. As this site is in the densely populated region on SW England this is most probably due to ozone losses by titration with NO emitted from vehicle exhausts (NEGTAP, 2001). Similarly the distributions of the deposition velocities and canopy resistances show typical log-normal distributions (Figure 5.7).

Despite the different time periods and vegetation types, the average and median total fluxes observed at each site are similar in magnitude. The two semi-natural canopies (Auchencorth Moss and Colt Park) tend to have slightly smaller fluxes than the grassland at Easter Bush and the oak forest at Alice Holt. The larger fluxes for the grassland during the summer months are due to the greater productivity which is associated with a larger stomatal conductance. (Figure 5.12); the measurements at Colt Park where made late in the season when the plants are less active and the canopy was very short. Fluxes of many trace-gases tend to be larger to forest canopies as they are rougher than short vegetation and so generate more turbulence; the oak trees also have quite high stomatal conductance in the summer months.

5.4.2 Diurnal and Seasonal Cycles

A diurnal cycle in ozone concentration is typically observed, and in rural areas of the UK this has a mid-afternoon peak and night-time minimum (Garland*, et al.*, 1979). The cycle is governed by both changes in the planetary boundary layer (PBL) and free troposphere, photochemical production and removal at the surface. During the daytime, turbulent mixing in the PBL, induced by both wind shear and thermal convection, lead to entrainment of ozone from the free troposphere. In sunny weather the presence of NOx and VOC emissions can also lead to photochemical production throughout the troposphere and so enhanced ozone concentrations. These processes produce an afternoon peak in ozone concentration when the atmosphere is most turbulent and UV

levels are at a maximum. During the night and early morning the lower regions of the boundary layer become thermally stratified and stable as the surface cools, greatly reducing entrainment of ozone from the free troposphere. The ozone concentration decreases rapidly as losses to dry deposition are not replenished by mixing from above and photochemical production cannot occur. The minimum concentration is usually reached between midnight and dawn.

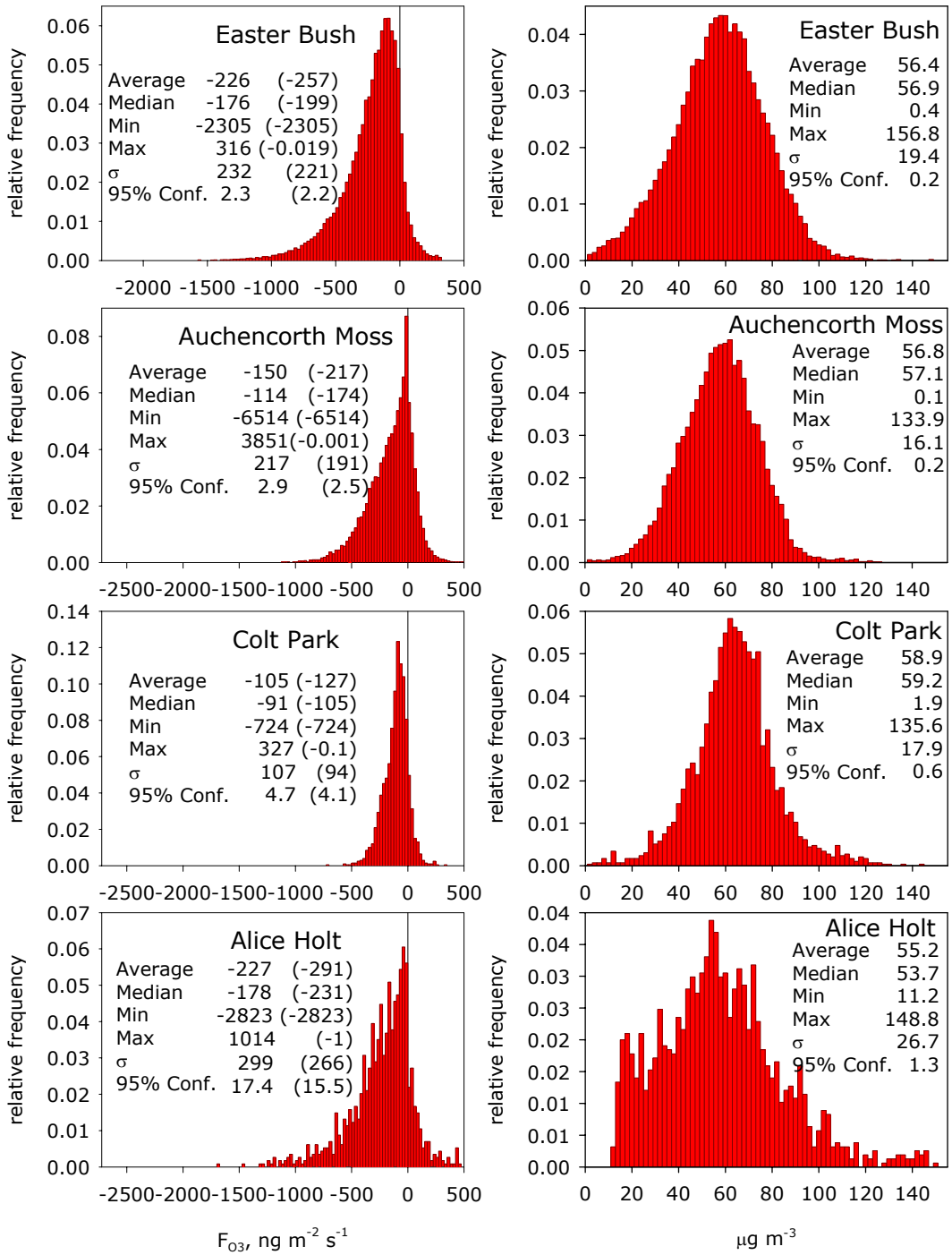


Figure 5.6. The relative frequency distributions of total ozone flux and concentrations measured at each monitoring site for their total monitoring periods. The values in brackets exclude positive fluxes.

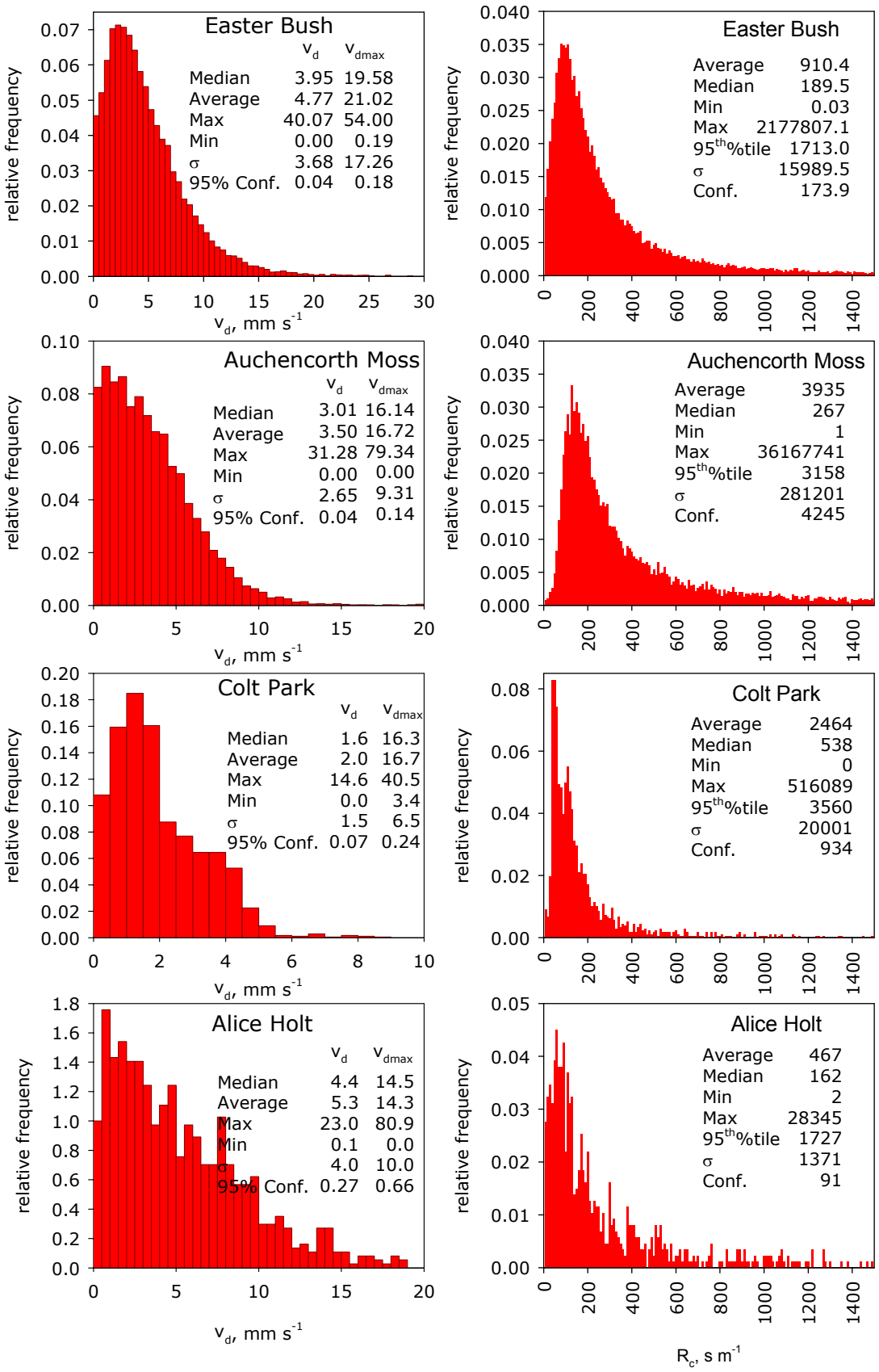


Figure 5.7. The relative frequency distributions of ozone deposition velocity and canopy resistance measured at each monitoring site for their total monitoring periods.

The PBL processes described above also are a large factor in controlling the ozone dry deposition rate and flux. When the atmosphere is more turbulent ozone is more readily transported down to the surface (small atmospheric resistances, R_a and R_b) and so fluxes tend to be larger whereas when the atmosphere is more stable deposition is suppressed and fluxes are smaller.

All four sites show typical diurnal cycles in ozone concentration, flux and deposition velocity (Figure 5.8) with mid-afternoon peaks and night-time minima. The diurnal cycles in ozone concentration are less pronounced at Easter Bush, Auchencorth and Colt Park than at Alice Holt as these sites are generally quite windy throughout the day whereas at Alice Holt the measurements occurred during a period of quite warm-sunny anti-cyclonic conditions when the atmosphere would have become very stable at night.

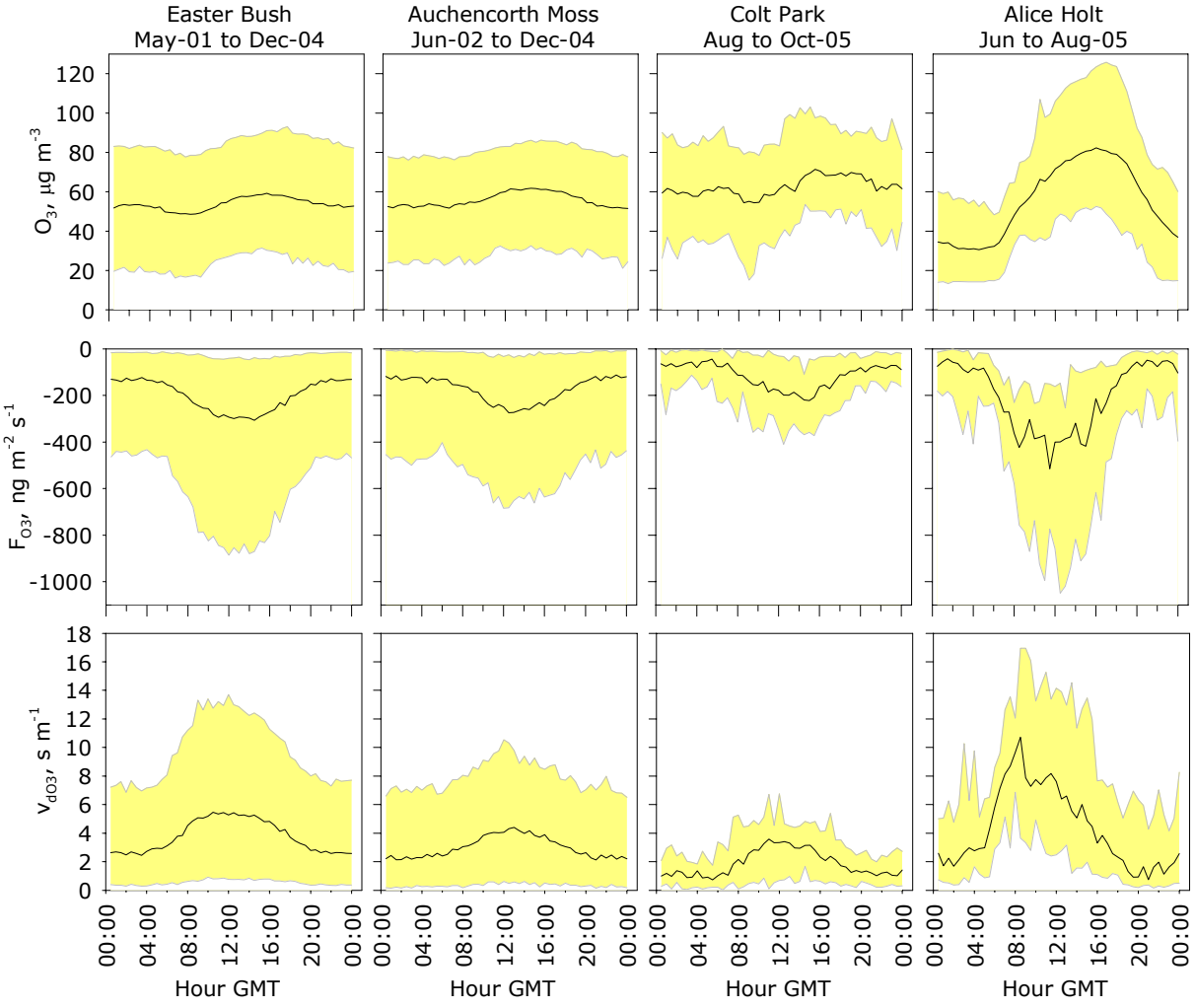


Figure 5.8. Diurnal cycles in the (top) ozone concentration, (middle) flux and (bottom) deposition velocity, the shaded area shows the 9th to 95th percentile range and the black line the median.

There is also a seasonal cycle in ozone concentrations and fluxes, driven by changing weather patterns, precursor emissions, and the growth cycle of vegetation. Concentrations typically show a spring/summer peak and winter minimum. The magnitude and length of the peak depends on the site type and its latitude: at clean

northerly sites a spring peak is typically observed which is typical of the northern hemisphere background ozone climate, while at more polluted southerly sites the peak extends into the summer due to regional ozone production.

Figure 5.9a shows a plot of the annual monthly averages from the top height at Easter Bush: in 2001 there is not enough data to comment on the form of the cycle; 2002 was particularly cold and wet so summer-time photochemical was suppressed, few episodes occurred and concentrations peaked in April which is typical of northerly background ozone concentrations; 2003 was very warm and fairly sunny so there was plenty photochemical ozone production which is reflected in the later peak in June; in 2004 concentrations were generally slightly higher than in the previous years but there were fewer episodes, indicating that this was due to background concentrations being enhanced over the whole region, this is also reflected in the peak occurring in April as this is typical of northerly background cycles. There is an upward trend in the monthly average concentrations of *ca*. 0.06 ppb y^{-1} which is typical or rural sites in the UK and Europe (NEGTAP, 2001, Simmonds et al., 2004; Vingarzan, 2004). It is possible that this trend is part of the general increase observed in background concentrations in the Northern hemisphere but more detailed analysis and comparison to other sites would be required to confirm this.

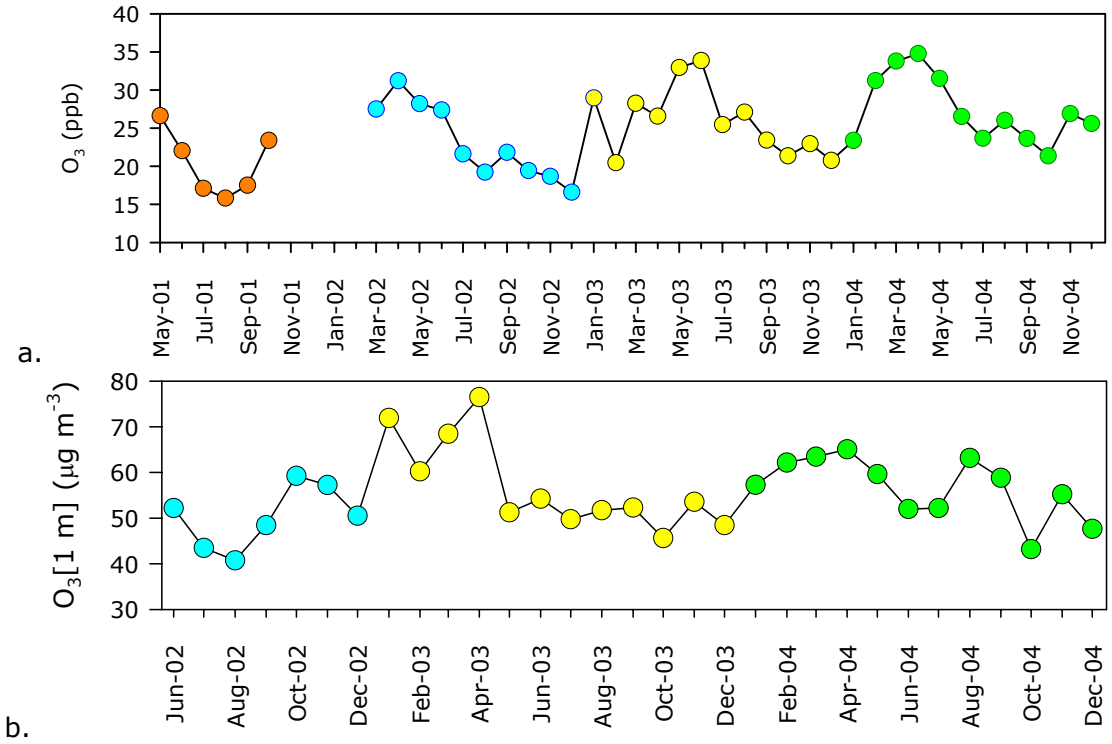


Figure 5.9 Monthly average ozone concentrations measured on (a) the top height at Easter Bush and (b) interpolated to 1 m above ground at Auchencorth Moss.

The seasonal cycles at Auchencorth for the period being considered here show similar patterns to Easter Bush but as this site is more remote from precursor emission sources

the spring peak is most prominent, particularly in 2003 (Figure 5.9b). There appear to be secondary peaks in the late summer/autumn of 2002 and 2004. As these are not observed at Easter Bush (only ~15 km north) they must be due to a local influence but it is unclear at present what that may be.

The ozone deposition velocity and total flux also exhibit a seasonal cycle although the peak in deposition tends to occur when the vegetation is most active and so stomatal uptake is at its maximum. The timing of the peak therefore depends on the land cover and seasonal weather conditions at the site. At Easter Bush the grass begins to get more active in April-May and continues to grow throughout the summer. Although the fields were harvested for silage and grazed throughout the measurement period the flux and deposition velocity follow a fairly smooth cycle with peaks in May to August or September (Figure 5.10). At Auchencorth, although there is some variation between years and months, there is also a fairly smooth cycle with a peak from April or May to July (Figure 5.11).

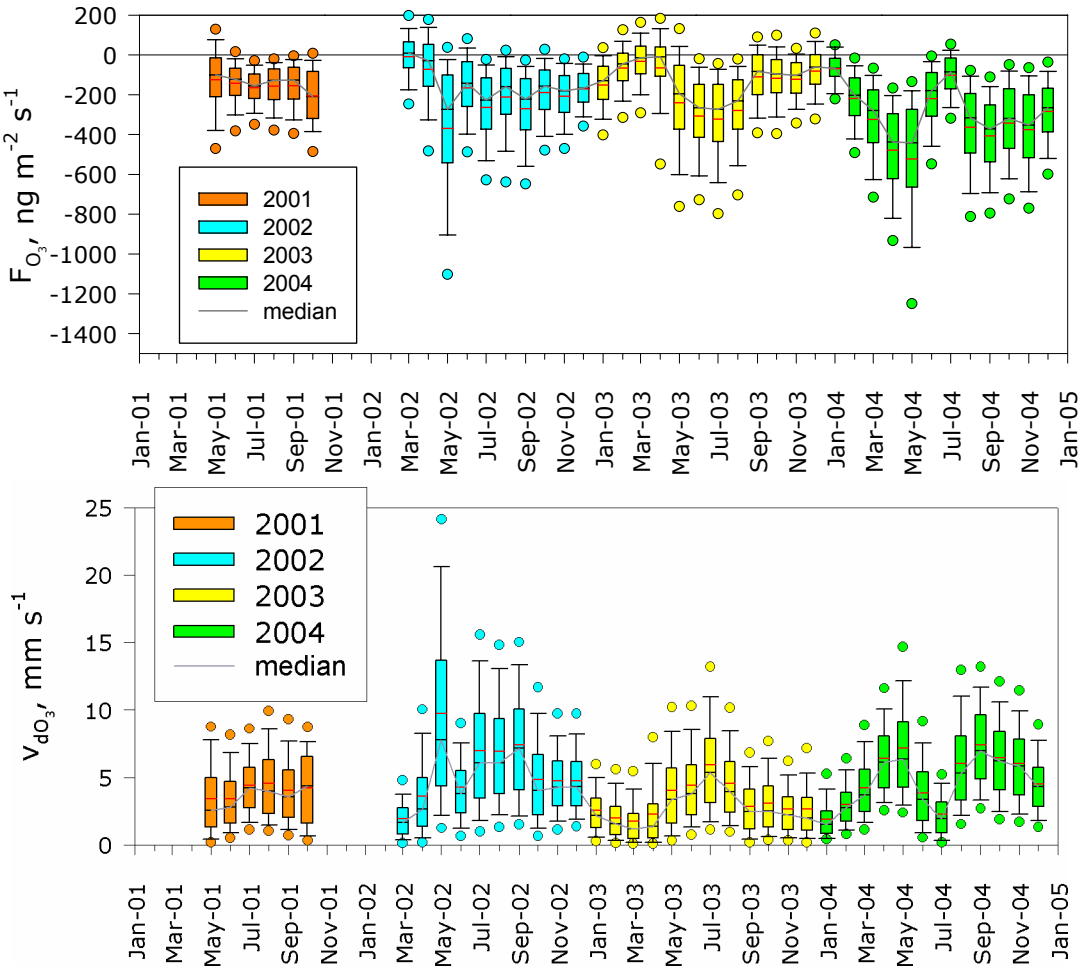


Figure 5.10 Monthly summary plot of total ozone flux and deposition velocity at Easter Bush: the box indicates the 75th and 25th percentiles; the black bar is the median; the red bar the average; the whiskers the 90th and 10th percentiles; the dots are the outliers at the 95th and 5th percentiles.

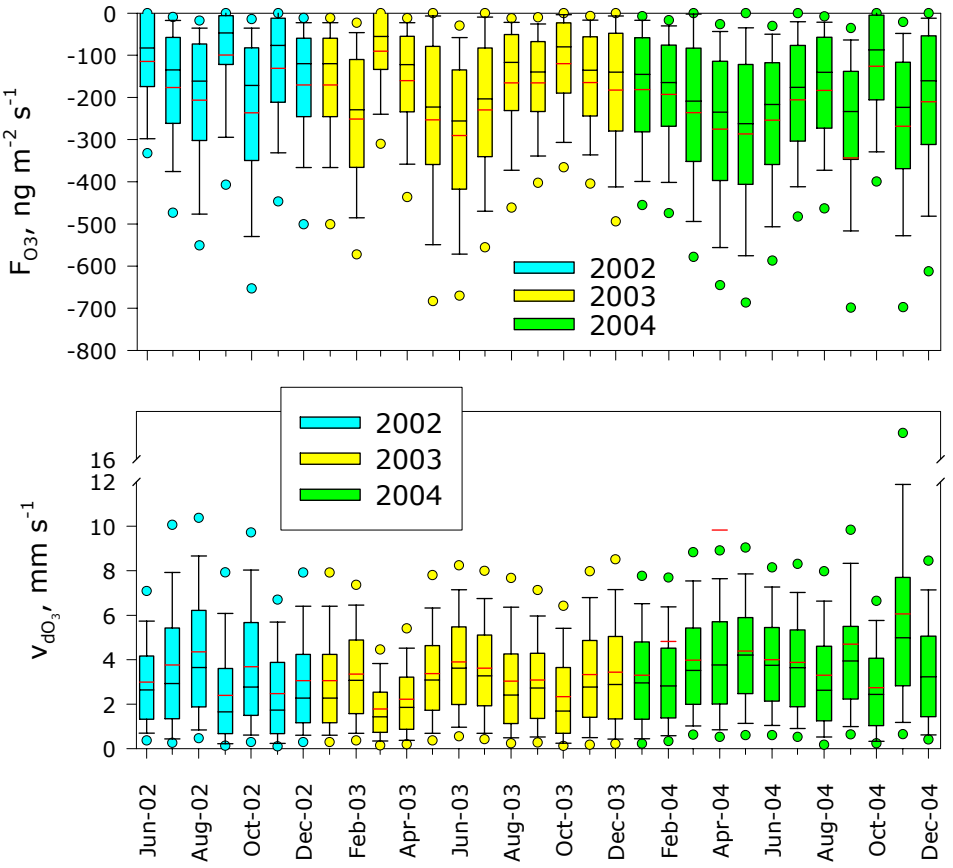


Figure 5.11 Monthly summary plot of total ozone flux and deposition velocity at Auchencorth Moss: the box indicates the 75th and 25th percentiles; the black bar is the median; the red bar the average; the whiskers the 90th and 10th percentiles; the dots are the outliers at the 95th and 5th percentiles.

5.4.3 Stomatal and Non-Stomatal Fluxes

The measurements of water-vapour flux were used to calculate the stomatal resistance and flux of ozone at Auchencorth Moss, Easter Bush and Colt Park during dry daylight conditions, as described in the Appendix. At Auchencorth Moss, Easter Bush and Alice Holt simple models of stomatal conductance were also used to extend the dataset to wet and night-time conditions. The non-stomatal component is then found as the residual of the total canopy and stomatal resistances (equation A15). The results are summarised in Table 5.7 which also gives the datacapture achieved at each site using measurements and models.

The plots in Figure 5.12 show the resulting time series for each site, with 0.5 hourly values for Easter Bush and Auchencorth Moss and daily medians for Alice Holt and Colt Park; Figure 5.13 shows the diurnal cycles in the fraction of the total flux that is stomatal. At Easter Bush and Auchencorth the stomatal term is often smaller than the non-stomatal. In particular at Auchencorth the stomatal flux is less than 50% of the non-stomatal flux for most of time (Table 5.6). Overall the stomatal flux is only ~6% of the total although it can be >60% on some days in the summer.

At Alice Holt the stomatal term is often the larger or only slightly smaller than the stomatal (median 50-60% of the total flux, Table 5.6). Although a model was used to gap-fill the time series most of the data occur during daylight hours (Figure 5.13) when the trees will have there stomata open, hence the stomatal flux should be significant. The days on which the non-stomatal flux is far greater, weather conditions (low sunlight, temperature or relative humidity) led to a reduction in stomatal conductance.

Table 5.7 Summary of stomatal and non-stomatal flux data. Values in brackets are recalculated from the flux and concentration results.

	F_s ng m^{-2} s^{-1}	F_{ns} ng m^{-2} s^{-1}	$O_3[z_{0'}]$ μg m^{-3}	R_s s m^{-1}	R_{ns} s m^{-1}	%Stom
Easter Bush						
Median	-41.7	-164.1	40.6	868 (974)	231 (248)	26 (20)
Average	-112.8	-203.1	41.6	1370 (369)	719 (205)	33 (36)
Max	0.0	-0.1	132.5	>10000	>10000	100 (23)
Min	-969.4	-1935.2	0.1	39 (0)	50 (0)	0 (33)
Stdev	131.3	164.2	16.9	3312 (-128)	2319 (-103)	27 (44)
pc05	-391.4	-527.4	15.1	103 (39)	68 (29)	4 (43)
pc95	-8.5	-16.4	70.8	2842 (8317)	2382 (4328)	86 (34)
%DC*	22.0 (32.2)					
Auchencorth Moss						
Median	-5.8	-111.3	48.8	865 (8363)	300 (438)	6 (5)
Average	-29.0	-149.9	48.7	1927 (1682)	1967 (325)	23 (16)
Max	-0.04	-0.01	116.3	>10000	>10000	100 (89)
Min	-343.8	-1098.1	0.6	244 (2)	20 (1)	0 (24)
Stdev	48.3	148.7	14.2	2245 (-293)	15473 (-95)	256 (25)
pc05	-141.0	-443.7	25.9	308 (184)	92 (58)	1 (24)
pc95	-1.6	-2.7	71.8	7192 (43651)	4944 (27047)	61 (38)
%DC	21.6 (57.7)					
Alice Holt						
Median	-323.5	-353.4	59.9	208 (185)	179 (169)	61 (48)
Average	-341.8	-867.2	63.8	518 (186)	504 (74)	59 (28)
Max	-3.2	-3.4	141.1	>10000	>10000	99 (48)
Min	-1015.0	-20492.2	27.7	82 (27)	4 (1)	2 (5)
Stdev	206.1	1861.9	19.8	1460 (-96)	1092 (-11)	24 (10)
pc05	-705.7	-3108.3	38.7	101 (55)	19 (12)	13 (19)
pc95	-33.4	-33.8	99.7	1378 (2982)	2035 (2946)	94 (50)
%DC	15.5 (20.0)					
Colt Park						
Median	-210.9	-58.4	64.8	289 (307)	1002 (1108)	77 (78)
Average	-221.2	-151.5	65.8	606 (297)	4906 (434)	73 (59)
Max	0.0	0.0	123.8	>10000	>10000	100 (0)
Min	-624.4	-15522.0	0.0	125 (0)	4 (0)	1 (4)
Stdev	104.5	937.3	16.1	2970 (-154)	23870 (-17)	20 (10)
pc05	-404.5	-246.1	42.6	182 (105)	259 (173)	36 (62)
pc95	-58.4	-5.2	92.9	857 (1591)	>10000	97 (92)
%DC	9.5 (15.9)					

*%data capture for total possible during measurement period and as percentage of total ozone flux measurements (in brackets)

At Colt Park the measured stomatal flux is larger than the non-stomatal (median ~80% of the total flux). This result was not expected as it was late in the season and the grass had been cut recently, hence the plants were not growing very much and stomatal conductance would be small. However it is possible that despite filtering for dry-surfaces the water-vapour flux may still contain a component that originates from the leaf surfaces or soil rather than the plant stomata and so the stomatal flux would be

overestimated. It was observed that even when the wetness sensor is reading completely dry there could still be significant water on the leaf surfaces as the sensor has different thermodynamic properties than the plants. This occurs at most sites on occasion but may have been more frequent at Colt Park due to its the damp upland climate. Thus the absolute values are to be treated with caution but the non-stomatal flux can still be used to look for controlling factors.

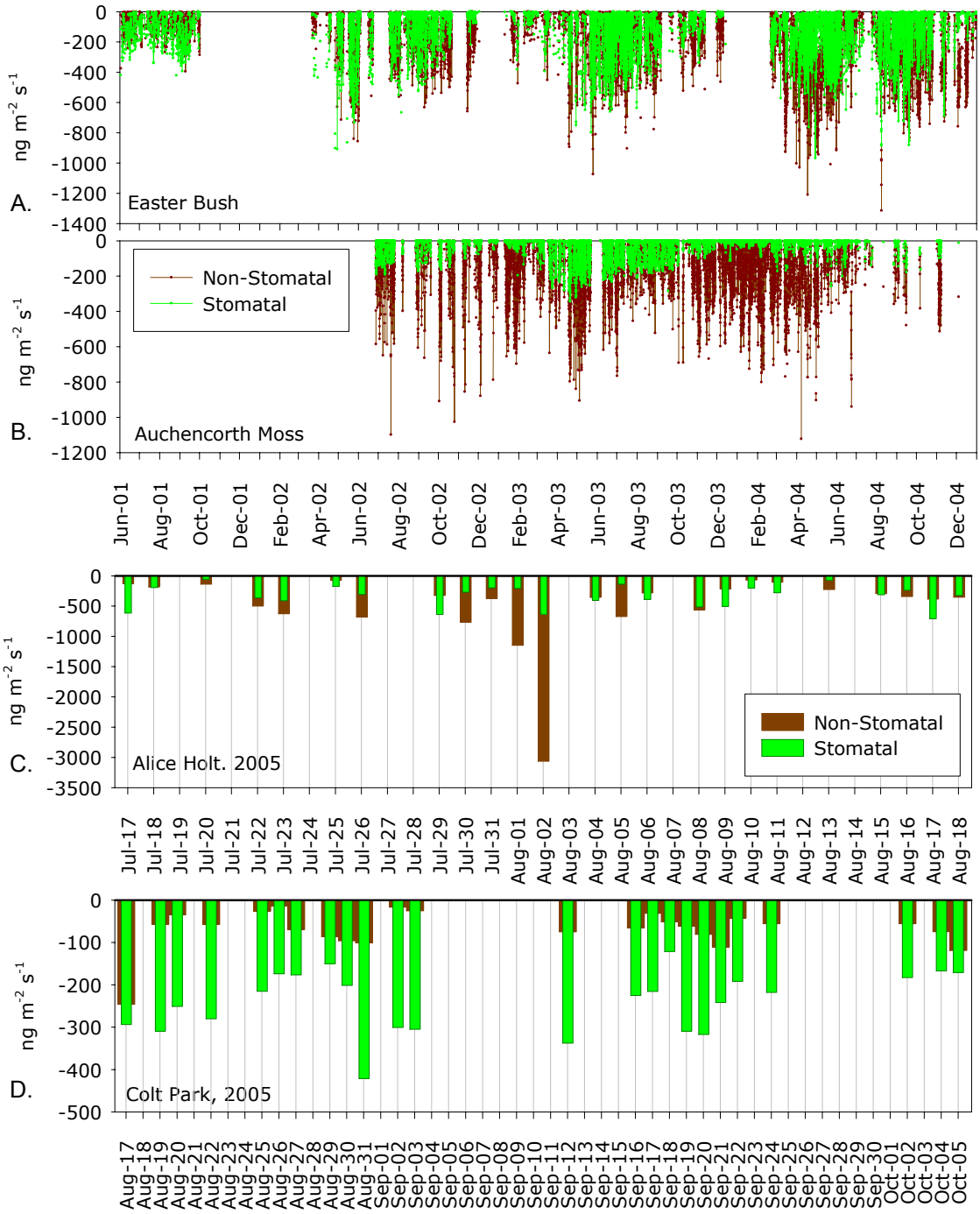


Figure 5.12 The stomatal and non-stomatal ozone flux at each site: A. Easter Bush and B. Auchencorth Moss, 0.5 hourly medians from measurements and models; C. Alice Holt, modelled daily medians; D. Colt Park, measured daily medians.

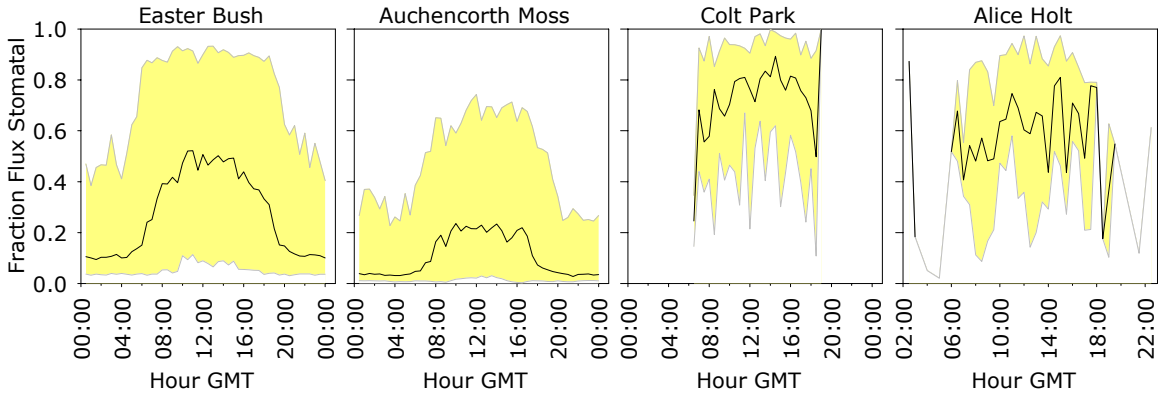


Figure 5.13. Diurnal cycles in the fraction of the total flux that is stomatal at each site, the shaded area shows the 9th to 95th percentile range and the black line the median.

5.4.4 Factors Controlling R_{ns}

Previous studies have identified surface temperature, solar radiation, friction velocity, surface wetness and relative humidity as possible controlling mechanisms on the non-stomatal flux (Coe *et al.*, 1995; Fowler *et al.*, 2001; Rondon *et al.*, 1993; Zhang *et al.*, 2002). Figure 5.14 shows scatter plots of R_{ns} at each site plotted against surface temperature ($T[z_{0'}]$), solar radiation (St), friction velocity (u_*) and relative humidity (RH, as a fraction). The data from Easter Bush and Auchencorth have been split into wet and dry conditions, while Colt Park is all dry-daylight data and there is no data currently available to split the Alice Holt results so it is considered separately; dry or wet conditions are selected:

- Dry – no rain and surface wetness reading totally dry, RH < 70%
- Wet – rain or surface wetness reading wet, RH >= 70%

Although there is a lot of scatter in the plots (Figure 5.14) some patterns an be seen: at all sites R_{ns} appears to decrease with increasing $T[z_{0'}]$, St and u_* for wet and dry surfaces; for dry surfaces R_{ns} decreases as RH increases but for wet surfaces a pattern is not clear; resistances may be slightly smaller for wet surfaces; values at Easter Bush and Auchencorth are of similar magnitude. To examine these trends more clearly the data are grouped by ranges of each control variable and medians calculated for the groups. For the purposes of curve fitting only points with a standard error (SE) less than 90% and at least 5 data in the group are included.

5.4.4.1 Surface Temperature

The plots in Figure 5.16 show the results of taking block medians of R_{ns} by every 1°C $T[z_{0'}]$ for dry (Figure 5.15a) and wet (Figure 5.15b) surfaces. For dry surfaces there is clearly a decrease in R_{ns_dry} with increasing temperature. At Easter Bush, which is the largest dataset, R_{ns_dry} appears to decline from ~600 s m^{-1} to ~200 s m^{-1} above 30°C where it levels off; hence an exponential decline is fitted ($R_{ns\_dry}(T_{z0'})$ = 146 + 359exp(-

$0.077T_{z0'}$), R^2 = 0.75, P < 0.001). At Auchencorth and Colt Park R_{ns_dry} also declines but there is not enough data to see if they follow the same pattern as Easter Bush. However linear regression shows that the rate of decrease is similar at Auchencorth and faster at Colt Park: -9.8, -7.2, -44.4 s m^{-1} $^oC^{-1}$ (R^2 = 0.7, 0.3, 0.6) at Easter Bush, Auchencorth and Colt Park respectively. The simplest interpretation of these results is that ozone is being destroyed at the surface by thermal decomposition, mediated by compounds on the leaf cuticle, although it is possible that it is also reacting with volatile organic compounds released from the leaf cuticle.

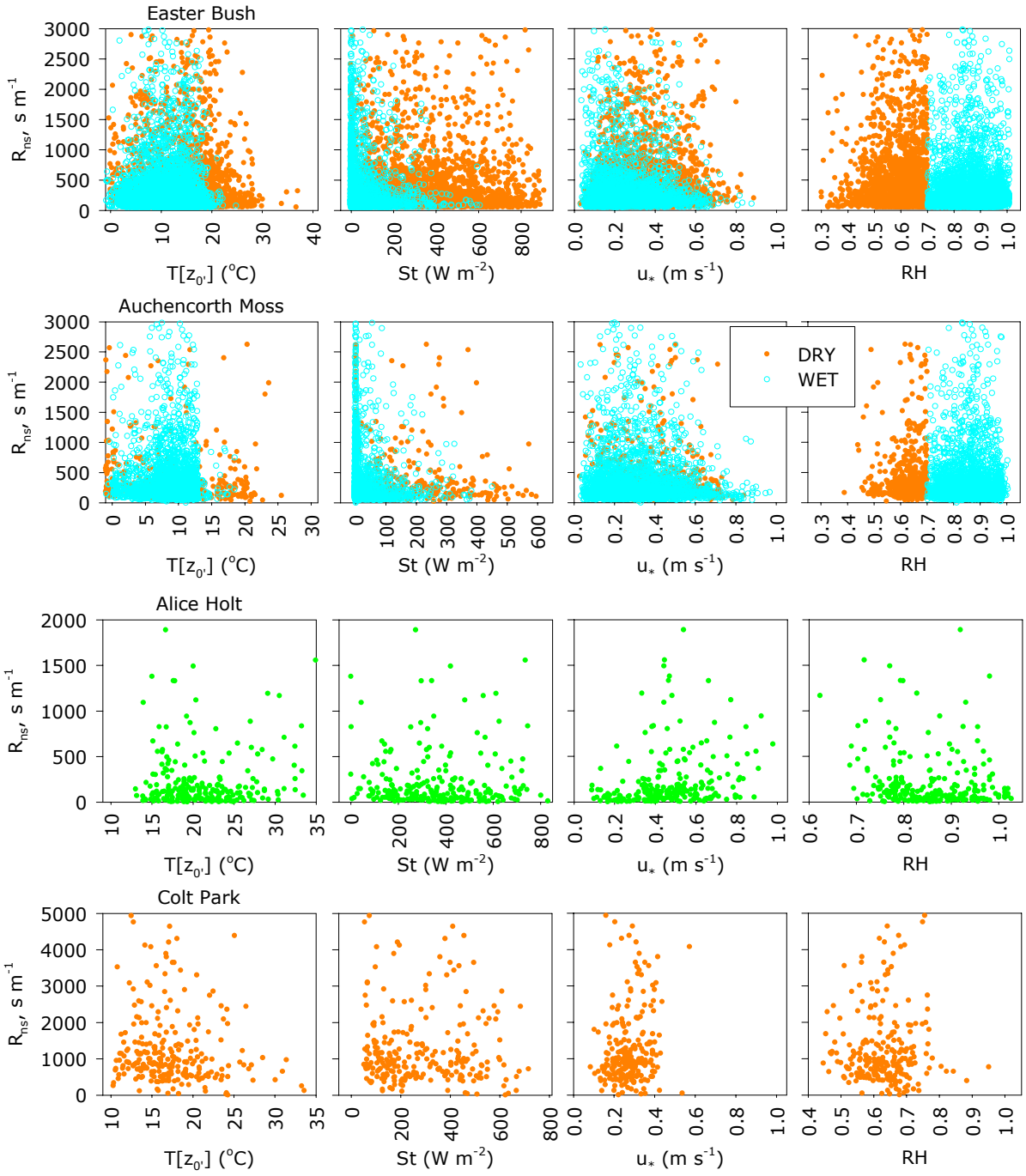


Figure 5.14. The variation in non-stomatal resistance (R_{ns}) with surface temperature ($T[z_{0'}]$), total solar radiation (St), friction velocity (u_*) and relative humidity (RH). Where possible the data have been split into wet (blue open circles) and dry (orange dots) surface conditions.

Data for wet surfaces are only available at Easter Bush and Auchencorth (Figure 5.15b). These data indicate that R_{ns_wet} may be constant at ~200 s m^{-1} (median 204 s m^{-1} at Easter Bush and 248 s m^{-1} at Auchencorth), although Easter Bush shows a slight decrease from ~300 to 200 s m^{-1} between 0 and 10°C and Auchencorth may do the same although there are too little data to be conclusive. It is usually assumed that ozone deposition rates to water will be very slow as ozone is not very soluble in water, for example Wesely *et al* (1981) estimated resistances of 3 to 8 x 10^5 s m^{-1} due to ozone dissolving or diffusing into water. However measurements over open water give values smaller than this (650 to 9000 s m^{-1};Gallagher*, et al.*, 2001;McKay*, et al.*, 1992;Wesely*, et al.*, 1981) and over wet vegetation some studies have reported enhanced deposition compared to dry conditions. These results are probably due to aqueous chemistry as ozone can be very reactive with some compounds in solution. For example ozone acts as an oxidising agent for dissolved SO_2 although the reaction is self-limiting and quickly saturates if pH is less than 6 however if the water pH is greater (due to sufficient NH_3 being present for example) the reaction can continue and represent a significant sink for ozone.

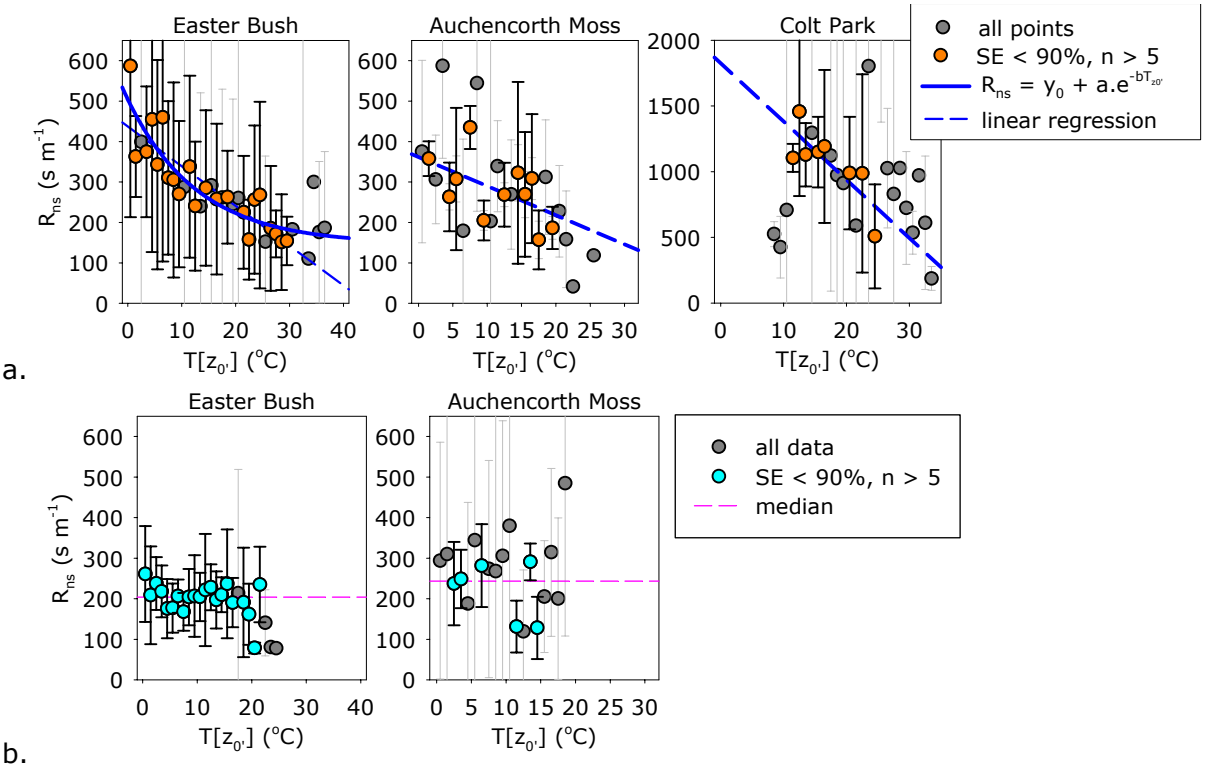


Figure 5.15 The variation in R_{ns} with $T[z_{0'}]$over wet and dry surfaces.

A simple model of the surface water chemistry (see Appendix for more details) predicts that with 35 ppb of O_3, 1 ppb of SO_2 and 1 ppb of NH_3 the pH of surface water would be ~6. This leads to ozone uptake rates of a few hundred ng m^{-2} s^{-1} depending on the thickness of the water layer, eg ~250 ng m^{-2} s^{-1} for 0.5 mm layer. If NH_3 concentrations are larger, 10 ppb, then the final pH is around 7, leading to uptake rates of several μg m^{-}

^{2} s^{-1}. However in practice, as ozone is relatively insoluable and the reaction rates very fast, uptake would be limited by diffusion within the liquid. For example, if only the top 10 μm contributed, the absolute removal rate would be only ~100 ng m^{-2} s^{-1} even at pH 7.2.

The Easter Bush data indicate that R_{ns_wet} is smaller than R_{ns_dry} up to temperatures of about 30°C where R_{ns_wet} is constant but R_{ns_dry} continues to decline. The data available for the other two sites do not cover the same range in temperature but are consistent with the results from Easter Bush.

5.4.4.2 *Solar Radiation*

The plots in Figure 5.16 show block medians of R_{ns} by every 25 W m^{-2} *St* for dry (Figure 5.16a) and wet (Figure 5.16b) surfaces. The results are similar to those for surface temperature in that resistances decline with increasing solar radiation. As these two parameters are closely related this is not unexpected and the relationship with *St* may simply be due to the effect of surface temperature. However, from the Easter Bush data, assuming 0°C ≅ 0 W m^{-2}, 25°C corresponds to ~1000 W m^{-2} (Coyle 2006) and plotting the sets of curves fitted to each shows that $R_{ns}(St)$ tends to give smaller values than $R_{ns}(T_{z0'})$ (Figure 5.17). This raises the possibility that photochemical or photolytic processes that remove ozone are also occurring on or near the leaf surfaces.

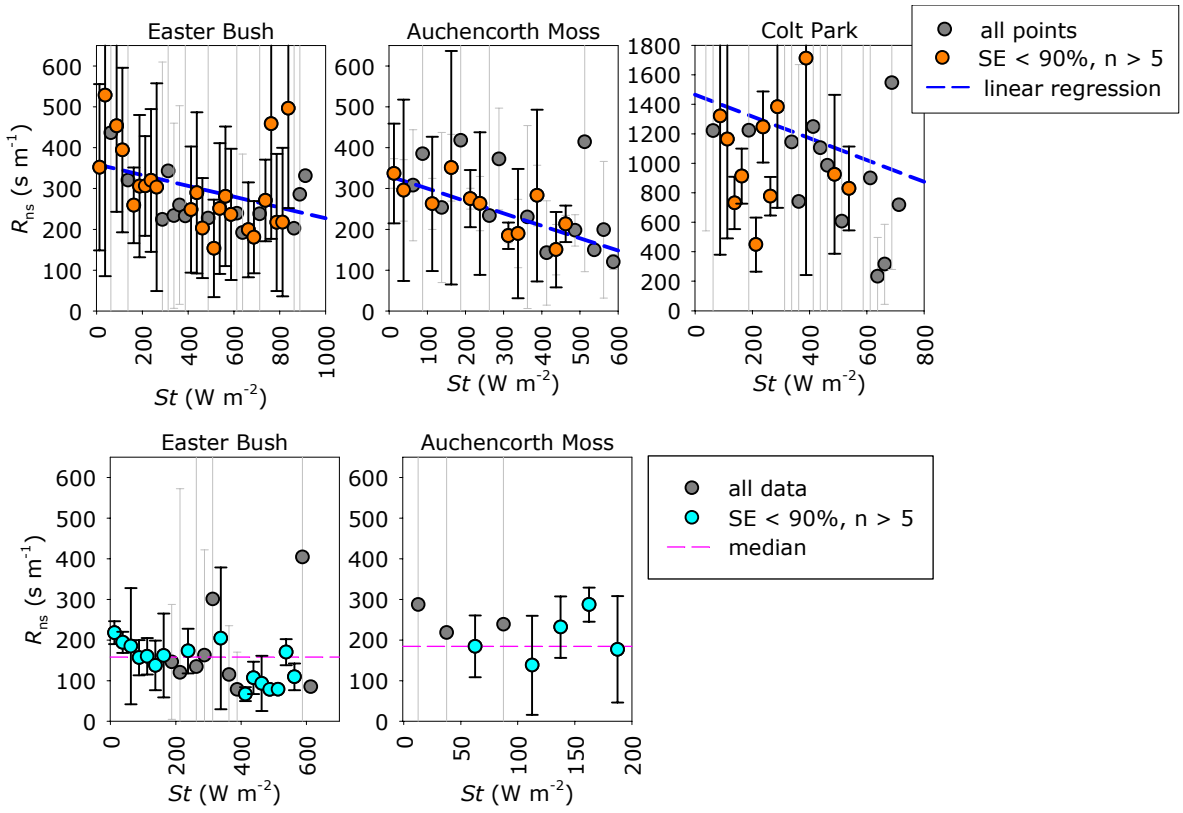


Figure 5.16 The variation in R_{ns} with *St* over wet and dry surfaces.

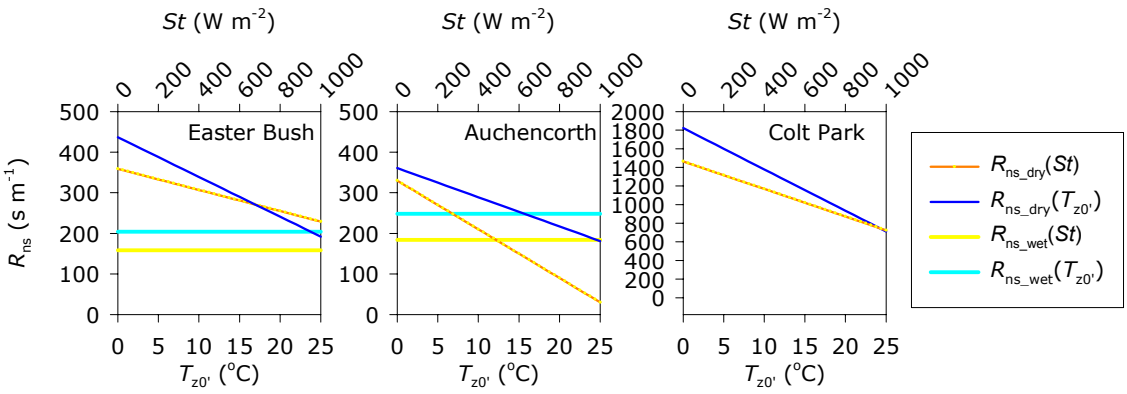


Figure 5.17 The fitted lines for the response of R_{ns} to $T[z_{0'}]$ and St over wet and dry surfaces.

5.4.4.3 Friction Velocity

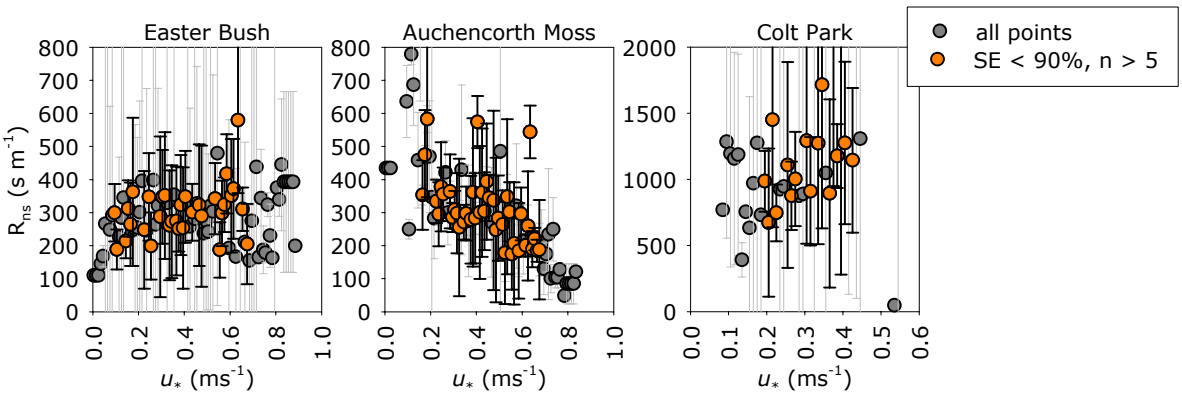
The plots in Figure 5.18 show block medians of R_{ns} by every 0.1 m s^{-1} u_* for dry (Figure 5.18a) and wet (Figure 5.18b) surfaces. Most models assume a decline in R_{ns} with increasing u_* or windspeed as the increased turbulence allows air to more deeply penetrate the canopy and so come into to contact with a greater surface area, however dry surfaces at Easter Bush and Colt Park do not show any dependence on u_* although Auchencorth does. At Colt Park the canopy was very short and so the maximum area was probably exposed at all times.

At Easter Bush the canopy went through several periods on growth, increasing from ~10 cm over the winter or after harvest up to 20-40 cm, with a corresponding increase in LAI from ~0.25 to ~4. Therefore we would expect to see a relationship between R_{ns_dry} and u_* at this site, although it may be masked by scatter due to the wide range of LAI (and SAI) the dataset covers. However restricting the data to a range of LAI (<1, 1.2 to 1.5, 2. to 2.4 for example) did not reveal any clear relationship. The grass canopy at Easter Bush is quite dense and when tall, tends to flatten in high winds. Therefore it is possible that it does not allow air to penetrate significantly and so the surface area exposed remains fairly constant.

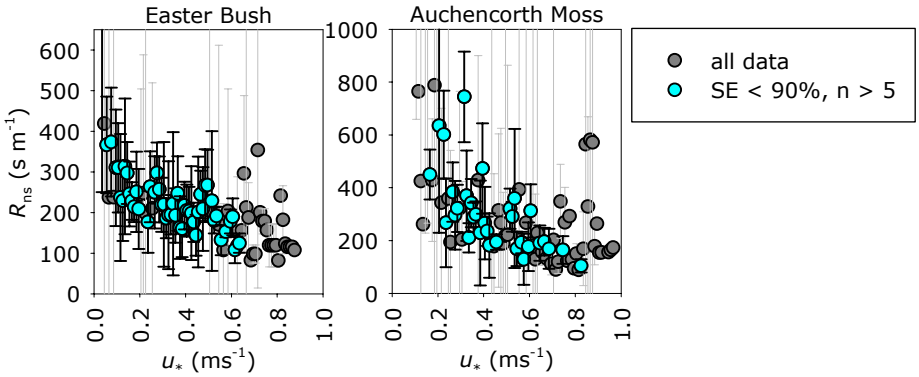
The canopy at Auchencorth is mixed with several grasses, forbs and other wild plants, some of which are more rigid than the *Lolium perenne* that dominates at Easter Bush. Its structure is therefore more open than the dense grass at Easter Bush and so it would more easily allow air to reach the lower canopy and ground surface, giving the observed response of R_{ns} to u_*.

Over wet surfaces both Easter Bush and Auchencorth show a clear relationship of R_{nswet} to u_*, with both decreasing from an R_{ns_wet} of around 500 s m^{-1} to about 100 s m^{-1} at 1 m s^{-1}. At Auchencorth this is again likely due to the open canopy structure but at Easter Bush the explanation may not be so simple as there is a the lack of a $R_{ns\_dry}(u_*)$ relationship. A simple model for aqueous ozone/SO_2 chemistry was proposed above to explain the decrease in R_{ns} with wet surfaces and it was stated that the reactions would

probably be limited to a thin layer on the surface of a water film. However if the film was agitated the thickness of the active water layer may increase and so reduce R_{ns_wet}.



a.



b.

Figure 5.18 The variation in R_{ns} with u_* over wet and dry surfaces.

5.4.4.4 SAI

Most models also assume that R_{ns} decreases with increases *SAI*[6] as there is a greater surface area available for ozone deposition. The measurements at Colt Park and Alice Holt took place over short periods when the *LAI* of each canopy did not vary significantly but at Easter Bush and Auchencorth the data cover several growing seasons. As described in the site descriptions, *LAI* at Easter Bush is estimated from the canopy height while at Auchencorth monthly values are assigned (from earlier work by Flechard *pers comm*.). Figure 5.19 shows plots of R_{ns} wet and dry versus *SAI* at each site. At Easter Bush there is no relationship for wet or dry conditions which supports the conclusion that the surface area of grass exposed to the air remains fairly constant in all conditions. At Auchencorth there is a slight decrease in R_{ns_dry} with increasing surface area but not for R_{ns_wet}. If the R_{ns_dry} data are restricted to points with 0.3 < u* < 0.4 m s^{-1} there is no longer a trend which indicates that although the more surface area may be exposed with faster friction velocities it does not increase as the canopy grows.

[6] LAI >= 1, SAI = LAI; LAI < 1, SAI = 1

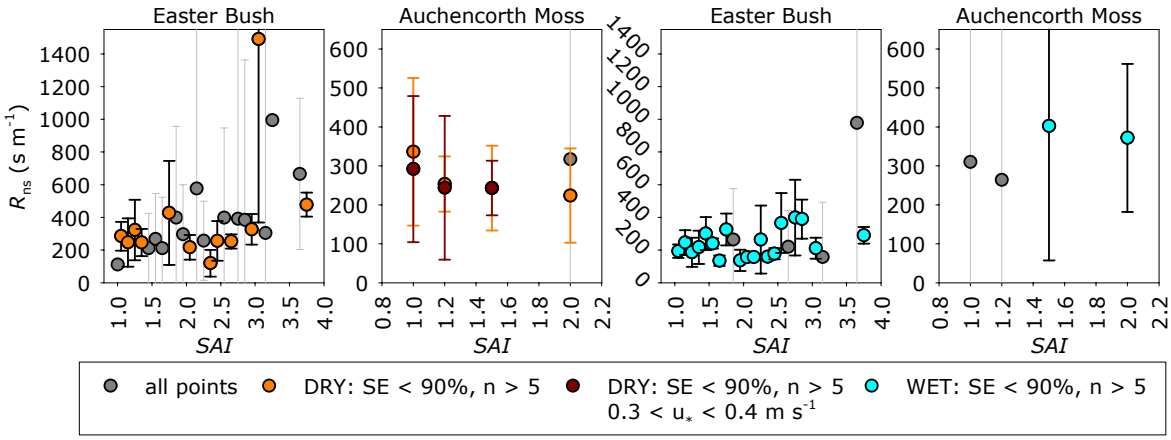


Figure 5.19 The variation in R_{ns} with *SAI* over wet and dry surfaces.

5.4.4.5 *Relative Humidity*

To allow the full range of *RH* to be examined the plots in Figure 5.20 show block medians of R_{ns} by every 2% *RH* for dry (Figure 5.20a) and wet (Figure 5.20b) surfaces where the *RH* condition for wet or dry has been neglected. On dry surfaces R_{ns_dry} clearly decreases with increasing *RH* above 60-70% but below this threshold it may decrease as *RH* decreases. A simple explanation for this pattern would be that as relative humidity increases over a dry surface a thin film of water builds up. Initially this would block the sites for thermal decomposition of ozone on the cuticle and so R_{ns} increases but as the film thickens aqueous ozone chemistry occurs and so R_{ns} decreases. Thus even though the wetness sensor indicates a completely dry surface, at high relative humidity there may be sufficient water present to allow aqueous processes to dominate. This emphasises the importance of getting good estimates of surface wetness and *RH* when examining the R_{ns} of vegetation.

On wet surfaces the data at Easter Bush indicate a slight decrease in R_{ns_wet} with increasing *RH*. However as the surface is already wet it is not clear why this is the case and no such trend is discernible at Auchencorth.

5.4.4.6 Alice Holt

As it is not possible to separate the Alice Holt data into wet or dry conditions the whole dataset is considered here and plotted in Figure 5.21. However relative humidity was always greater than 60% so it is likely that there was some water present on non-stomatal surfaces.

The plot of R_{ns} versus $T[z_{0'}]$ (Figure 5.21a) shows very little change in R_{ns} with temperature but as all surface conditions are included this is not surprising. Easter Bush and Auchencorth both show far weaker $R_{ns}(T[z_{0'}])$ relationships when the data are considered as a whole (Figure 5.22). There is a slight decrease in R_{ns} with *St* (Figure 5.21b) which supports the idea that photochemical/photolytic processes are occurring, as a relationship was found on both wet and dry surfaces at the other sites. Alice Holt also

shows little change in R_{ns} with u_*, however as it is a forest canopy which normally allow air to penetrate as wind speeds increase this result cannot be easily explained. In the case of *RH*, R_{ns} clearly declines as *RH* increases above 60% as observed at the other sites.

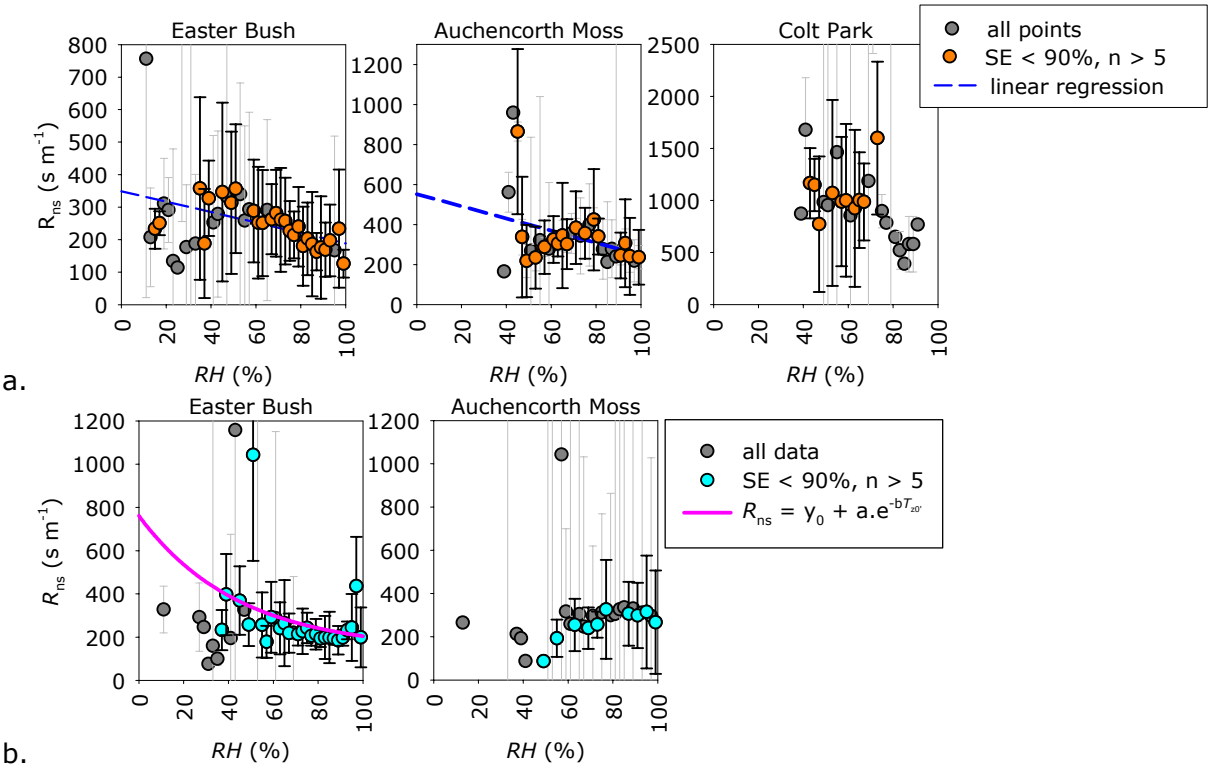


Figure 5.20 The variation in R_{ns} with relative humidity (*RH*) over wet and dry surfaces.

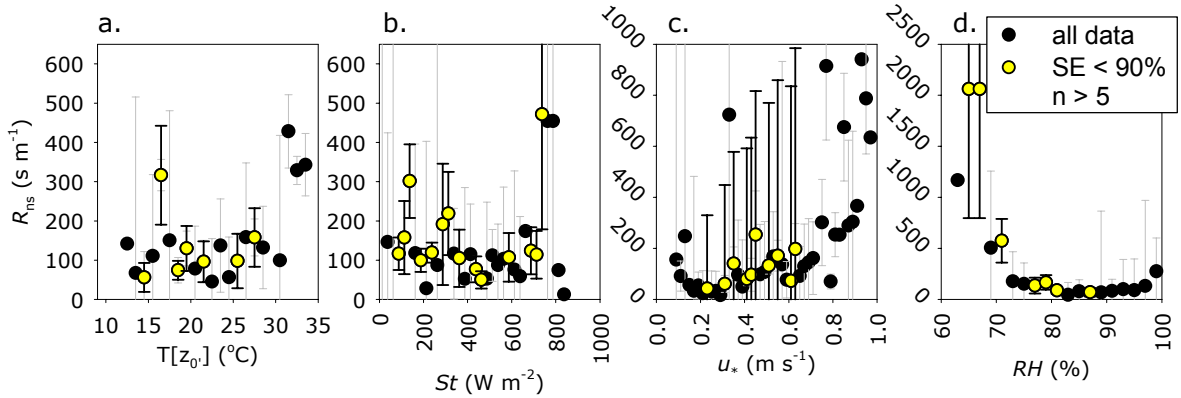


Figure 5.21 The variation in R_{ns} with $T[z_{0'}]$, *St*, u_* and *RH* at Alice Holt.

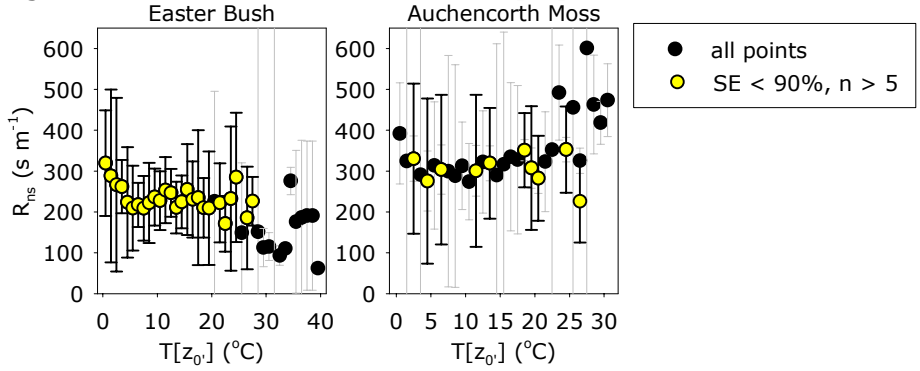


Figure 5.22 The variation in R_{ns} with $T[z_{0'}]$ for all surface conditions.

5.5 Conclusion

A substantial dataset of ozone flux measurements for an upland moor/bog (Auchencorth Moss) and improved grassland (Easter Bush) has been compiled, as well as shorter periods of measurements over an oak forest (Alice Holt) and semi-natural upland grassland (Colt Park). The data include estimates of canopy conductance and so stomatal ozone uptake, a larger value of conductance leads to more uptake.

The sites with the greatest total ozone deposition are Alice Holt and Easter Bush. At the former, the trees tend to have a large stomatal conductance during the summer to maintain their water balance and the roughness of the tree canopy causes more turbulence and so enhances atmosphere/canopy exchange. At the latter, this is due to the grassland (mainly *Lolium perenne*) being highly productive with an associated large stomatal conductance during the growing season. This would increase its ability to out-compete other species at higher ozone concentrations given it is not very sensitive to ozone compared to some other grasses, as was noted in Section 4.

Of all four sites, Colt Park has the smallest total ozone deposition as it was late in the season after the grass had been cut so it was short and not very productive. It was therefore anticipated that the stomatal flux would be a small fraction of the total (10-20%) but the measurements indicated much larger values of 70-80%. However it is likely that there was some interference from non-transpired water-vapour which would have increased the estimates of stomatal flux. Although a wetness sensor was used to indicate wet or dry surface conditions it does not accurately mimic the plant surface and may have been dry even when the canopy was wet. A longer measurement period and calculation of dew duration using an energy balance model would improve results for a damp upland site such as this.

Although it has been recognised that non-stomatal ozone deposition is influenced by factors such as surface temperature, solar radiation and humidity most models assume it is constant and varies with solely with *LAI* and in some cases wind speed. In order to improve understanding of the non-stomatal deposition process the variation in non-stomatal canopy resistance (R_{ns}) with several variables was examined at each site. The R_{ns} estimates show some response to surface temperature, solar radiation, friction velocity and relative humidity depending on surface conditions. Overall it appears that:

- Dry Surfaces: R_{ns} declines with increases in both $T[z_{0'}]$ and *St* which supports the hypothesis that ozone is destroyed on leaf surfaces by thermal decomposition, mediated by compounds on or emitted by the leaf surface and that some photochemical/photolytic reactions also occur. Ozone may also be reacting directly with volatile organic compounds emitted by the canopy although

measurements of VOC emissions to date have not found any compounds that react quickly enough with ozone to cause the ozone fluxes observed.

Although only dry surfaces were selected it is likely that there will be some surface water present on the leaves when the relative humidity is above 40-60%. This appears to inhibit the reactions described above as R_{ns} increases, although at higher humidity it again begins to decrease suggesting that a sufficiently thick water film has built up for the wet surface reactions to occur.

- Wet Surfaces: R_{ns} is smaller over wet surfaces than it is over dry indicating the water films on vegetation are an efficient sink for ozone. A slight decrease in R_{ns} with both $T[z_{0'}]$ and *St* is detectable at Easter Bush and so it is assumed that there are aqueous chemical reactions occurring and that additional photochemical reactions may occur in daylight. A simple model of $O_3/SO_2/NH_3$ showed that if sufficient NH_3 was present to increase the pH of a water film above 7 then SO_2/O_3 oxidation could be a significant sink for ozone.

Although R_{ns} would be expected to decline with increasing u_* and *SAI* this trend was only observed at Auchencorth Moss in both wet and dry conditions. It was concluded that the surface area exposed to ozone does not vary significantly if the canopy is quite dense and tends to flatten in high winds, such as at Easter Bush. Any periods when turbulence brought air into the canopy and down to the soil must be too brief or infrequent to be detectable in these data.

Appendix – Overview of Methods Used to Process the Data and Calculate Fluxes

As a first step any known periods where an instrument was faulty or was being calibrated are removed, any calibrations are then applied and the data set visually examined for outliers. For the application of micrometeorological analysis some criteria have to be met for the data to be valid, mainly: the wind direction must be from the area of good fetch and turbulence must be well developed. The first is simply ensured by checking the wind direction (site dependant, Table 5.A1), while for the second the integral turbulence characteristic (*ITC,* equation A1) is used to test for sufficient turbulence.

$$ITC = \left| \frac{\left(\frac{\sigma_w}{u_*}\right)_{model} - \left(\frac{\sigma_w}{u_*}\right)_{measurement}}{\left(\frac{\sigma_w}{u_*}\right)_{model}} \right| \qquad \text{(A1)}$$

$$\left(\frac{\sigma_w}{u_*}\right)_{model} = 0.21 \cdot \ln\left(\frac{z_+ \cdot \angle}{u_*}\right) + 3.1 \qquad z_+ = 1\,m \qquad \text{(A2)}$$

where σ_w = standard deviation of the vertical wind speed component
u_*= friction velocity, see equation A4
$\angle$ = Coriolis parameter = $2\Omega\sin\lambda$
Ω = angular veocity of the Earth = 7.2921×10^{-5} rad s^{-1}
λ = site latitude (radians)

Where the ITC is less than 30% well developed turbulence can be assumed. Some basic parameters and the flux of ozone are calculated as follows, depending on the methods being used (Table 5.A1). The horizontal and vertical components of the wind-speed (*u, v* and *w* respectively) can be equated to a mean over time plus the instantaneous departure from the mean, commonly written:

$$u = \bar{u} + u' \qquad \text{(A3)}$$

where $\bar{u}$ = mean with time, u' = instantaneous deviation from the mean value

The sonic anemometers measure the three components of wind-speed as well as the air temperature and these can be used to calculate parameters such as the friction velocity (u_*) and sensible heat flux (H).

Eddy Correlation

$$u_* = (-\overline{u'w'})^{0.5} \qquad \text{(A4)}$$

where u' and w'= instantaneous wind speed deviations of the parallel horizontal and vertical components of the wind flow from their mean values of wind speed respectively

Sensible heat flux, $H = \rho c_p \overline{w'T'}$ (A5)

Latent heat flux, $\lambda E = -\rho\lambda \overline{w'q'}$ (A6)

$$F_{O3} = \overline{w' |O_3|'} \quad \text{(A7)}$$

Ozone Gradient

$$F_{O3} = -k\, u_* \frac{\partial |O_3(z-d)|}{\partial ln(z-d) - \psi_H} \quad \text{(A8)}$$

where k = von Karman's constant = 0.41, $|O_3(z\text{-}d)|$ = ozone concentration at height z-d, z = measurement height, d = zero plane displacement, ψ_H = stability correction function for sensible heat flux

In stable conditions

$$\psi_H [\zeta] = -5.2\, \zeta = -5.2 \frac{z-d}{L} \quad \text{(A9)}$$

In unstable conditions Paulson, 1970:

$$\psi_H [\zeta] = 2 ln\left(\frac{1+\chi^2}{2}\right) \quad \text{(A10)}$$

where $\chi = (1\text{-}16\zeta)^{0.25}$

Full details can be found in the following references: Businger*, et al.*, 1971;Lee*, et al.*, 2004;Monteith*, et al.*, 1990;Sutton*, et al.*, 1993;Thom, 1975;Wilczak*, et al.*, 2001.

At all sites some periods of positive ozone fluxes are measured (Figure 5.8) although in principle ozone is never emitted from the surface. When ozone concentrations are low the measurements become more uncertain and so some of the positive fluxes are due to this. In some circumstances atmospheric conditions may lead to the measurement of an apparent upward flux. For example, Figure 5.A1 shows the total uncertainty in the Easter Bush flux measurements for individual 0.5 hourly values and 25 ng m^{-2} s^{-1} block medians; when fluxes are small or positive the uncertainty is greatest. In the analysis of canopy resistance and deposition velocity periods of positive fluxes are removed.

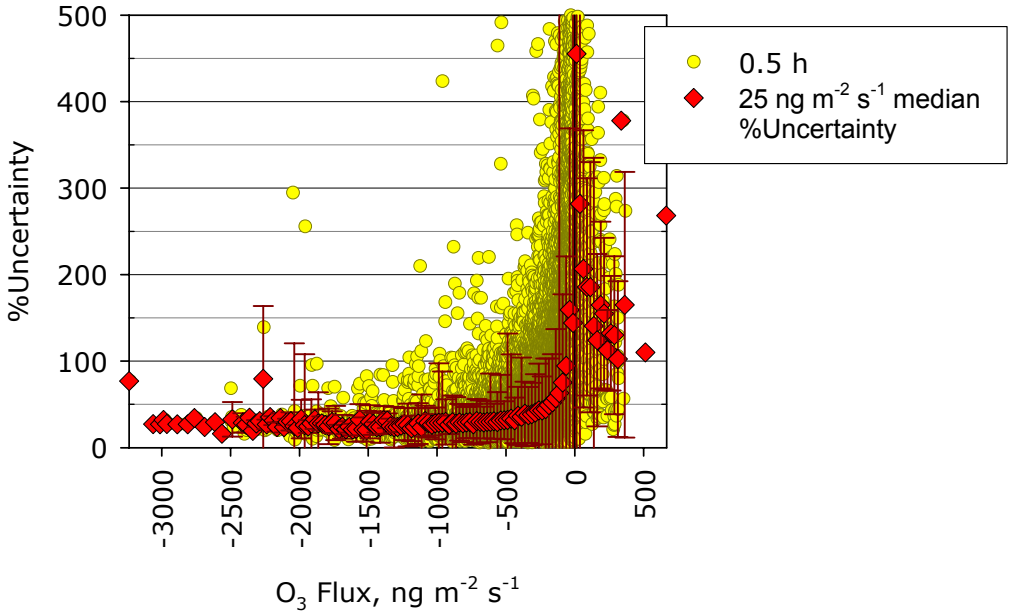


Figure 5.A1 Total uncertainty in measurements of ozone flux at Easter Bush.

Measuring and Modelling Stomatal Fluxes

If transpiration from vegetation is the only source of water vapour from the surface, ie the surface is completely dry and stomata are open, then the bulk-canopy stomatal resistance to water-vapour transfer can estimated using: canopy surface temperature ($T_{z0'}$); vapour pressure at height $d + z_{0'}$, e($z_{0'}$); R_a; R_b; λE and H (Coe et al., 1995):

$$T[z_{0'}] = T[z\text{-}d] + \frac{H}{\rho c_p}(R_a[z-d] + R_b) \qquad \text{(A11)}$$

$$e[z_{0'}] = e[z\text{-}d] + \frac{Ep}{\rho\varepsilon}(R_a[z-d] + R_b) \qquad \text{(A12)}$$

$$R_{c1w} = \frac{\rho\varepsilon}{p}\frac{e_s[T[z_{0'}]] - e[z_{0'}]}{E} \qquad \text{(A13)}$$

where z = reference height (m), d = zero plane displacement height (d), $z_{0'}$ = roughness length for dissipation of heat and trace-gases, ρ = air density (kg m^{-3}), p = atmospheric pressure (kPa), ε = ratio of the molecular weight of water to that of dry air ≈ 0.62, $e_s(T_{z0'})$ is the saturation vapour pressure at $T_{z0'}$

Assuming ozone has zero mesophyll resistance, its stomatal resistance can be calculated by scaling R_{c1w} for the difference between the molecular diffusivity of ozone and H_2O:

$$R_{c1w}D_w = R_{c1O3}D_{O3} \text{ where } D = \text{molecular diffusivity}, D_w/D_{O3} = 1.51 \qquad \text{(A14)}$$

Following the standard resistance analogy (Chamberlain, 1966; Monteith and Unsworth, 1990) the non-stomatal resistance can then by found using:

$$R_{ns} = \left(\frac{1}{R_c} - \frac{1}{R_{c1O3}}\right)^{-1} \qquad \text{(A15)}$$

where R_c total canopy resistance to ozone; $R_c = \frac{|O_3|(z-d)}{F_{O3}(z-d)} - (R_a + R_b)$ and $|O_3|$ = ozone concentration.

As the measurements of R_{c1} can only be made in dry-daylight conditions the data-capture achieved is small (typically <10% compared to >70% for other parameters). To extend the conditions during which R_{ns} can be examined stomatal resistance (conductance, $g_s = 1/R_{c1}$) models are used.

Table 5.A1. Wind sectors, time step and methods employed at each site

	Auchencorth Moss	Easter Bush	Colt Park	Alice Holt
Wind sectors excluded	60 - 170°N	130 – 150°N, 305 – 315°N	8 – 160 °N	0
Time step	15 minute averaged to 0.5 hour	0.5 hour	0.5 hour	0.5 hour
Methods used:				
Ozone Flux	Gradient	Gradient	Eddy-correlation	Eddy-correlation
Turbulence	Eddy-correlation	Eddy-correlation	Eddy-correlation	Eddy-correlation

Stomatal Resistance Models

There are two approaches that have commonly been used to model stomatal resistance, multiplicative or photosynthesis. In either scheme the stomatal resistance for a sunlit leaf-level is usually estimated then scaled up to the bulk-canopy by estimating the degree of canopy shading for example. Where R_{ns} is of interest, only the bulk-canopy resistance is required so simple site-specific models can be derived from measurements of canopy water-vapour flux. Such models were developed using the multiplicative method for Auchencorth Moss, Easter Bush and Alice Holt and also a photosynthesis model at Easter Bush. The models applied at each are outlined below:

Auchencorth Moss

Stomatal resistances for ozone were parameterized for Auchencorth Moss from R_{c1} values measured during 1995 (Fowler *et al* 2001), following a scheme similar to that of Hicks *et al*. (1987):

$$R_{c1\_O3} = 1.51\left[\frac{R_{\min}(1 + b/S_t)}{f_e f_T f_w}\right] \quad \text{(A16)}$$

where S_t is global solar radiation (W m_{-2}), f_e, f_T and f_w are correction factors for the effects on stomatal opening of humidity, temperature and water potential, respectively, while the parameters R_{min} and b are derived from the measurements. Table 5.A2 summarises the parameters and functions used.

Table 5.A2 Parameters for the Auchencorth Model

R_{min}	98			
b	393			
$f_T = \left[\frac{T - T_{min}}{T_{opt} - T_{min}}\right]\left[\frac{T_{max} - T}{T_{max} - T_{opt}}\right]^{\left[\frac{T_{max} - T_{opt}}{T_{opt} - T_{min}}\right]}$	T_{min} 2	T_{opt} 20	T_{max} 45	
$f_e = 1 - 0.1 b_e e_s(T_s)[1 - RH]$	b_e 0.1			
As the site is normally well watered, f_w	1			

Easter Bush

a) JMod

Multiplicative schemes which are based on empirical relationships between stomatal resistance and environmental variables (Jarvis, 1976). The model takes the form shown below (7) where g_{max}, the maximum stomatal conductance is scaled to account for the influence of plant age and several environmental variables, solar radiation, temperature, vapour pressure deficit, soil-water potential and leaf-area index.

$$R_{c1\_O3J} = 1.51\left[\frac{g_{max}.f_{pot}(max\,imum(f_{min}, (f_{light}.f_T, f_{vpd}, f_{SWP}, f_{LAI}))}{a_w}\right]^{-1} \quad \text{(A17)}$$

where g_{max} = maximum stomatal conductance, f_x = scaling factors described below, a_w is the conversion factor for mol m^{-2} s^{-1} to m s^{-1}

Standard forms of the scaling functions are used (Emberson et al., 2000; Coyle, 2005) and parameterised using the measured values of R_{c1w} as summarised in Table 5.A3.

b) BMod

"Ball-Berry type" photosynthesis schemes which are more mechanistic but remainsemi-empirical, based on the relationships between physiological parameters such as net photosynthesis and carbon dioxide concentration (Ball et al., 1987). A simple equation can be used to describe the response of stomatal conductance to the net rate of CO_2 uptake (A_n), the relative humidity (as a ratio, RH_s) and CO_2 concentration at the leaf surface:

$$R_{c1\_O3B} = 1.51\left[\left(m\frac{(A_n - A_s - R_d)RH_s}{|CO_2|(z_{0'})} + b\right)\Big/ a_w\right]^{-1} \quad \text{(A18)}$$

where A_n is the measured CO_2 flux, As is the estimated soil respiration and R_d = 3.5 μmol-CO_2 m^{-2} s^{-1}, $|CO_2(z_{0'})| = |CO_2(z\text{-}d)| + A_n(R_{a(z\text{-}d)} + R_{bCO2})$ = CO_2 concentration at the leaf surface.

The parameters m and b are the linear regression coefficients obtained from a plot of the photosynthesis ratio term with measurements to stomatal conductance ($1/R_{c1w}$), as given in Table 5.A3.

Table 5.A3 Parameters for JMod and BMod

a) JMod			
g_{max}	985 mmol m^{-2} s^{-1}		
f_{min}	0.033		
f_{light}= (1-exp$^{-\alpha PAR}$)	α = -0.002		
$f_T = \left[\frac{T - T_{min}}{T_{opt} - T_{min}}\right]\left[\frac{T_{max} - T}{T_{max} - T_{opt}}\right]^{\left[\frac{T_{max} - T_{opt}}{T_{opt} - T_{min}}\right]}$	T_{min} -1	T_{opt} 18	T_{max} 40
f_{vpd} step function: linear decline to f_{min} when vpd_{min} <vpd< vpd_{max}	vpd_{min} 0.4		vpd_{max} 2.5
f_{SWP} step function: linear increase from f_{min} when SWP_{min} <vpd< SWP_{max}	SWP_{min} -800		SWP_{max} -100
f_{LAI} step function: linear decline to f_{min} when LAI <LAI_{max}	LAI_{max} 2		
f_{pot} step function: linear increase from f_{potmin} to 1 from day no. 1 to 91; linear decrease from 1 to f_{potmin} from day no. 274 to 365; f_{potmin} = 0.5			
b) BMod			
m = 24537 b = 418 R^2 = 0.95			
R_d = 3.5 µmol-CO_2 m^{-2} s^{-1}			
A_s, from measurements: ~2 µg-CO_2 m^{-2} s^{-1} in the winter to 10 µg-CO_2 m^{-2} s^{-1} in August (Jan to Dec: 2, 2, 3, 5, 7, 8, 9, 10, 7, 3, 2, 2)			

Testing of each model showed that each performed reasonably well and none was clearly better that the other at predicting stomatal conductance. The measured time series of R_{c1_o3} was therefore gap filled using BMod in preference to JMod as in principle BMod is using a more mechanistic approach and so should better reflect reality.

Alice Holt

At Alice Holt a simple multiplicative model was fitted to measurements of bulk-canopy water-vapour flux made during previous years (Broadmeadow *pers com*). The model and its parameterisation are summarised below in equation A19 and Table 5.A4. As the measurements of water-vapour flux were not currently available for the period examined in this report only modelled data are used to calculate R_{ns}.

$$R_{c1\_O3} = 1.51\left[\frac{g_{max}.f_{PAR}f_{vpd}f_w f_u}{a_w}\right]^{-1} \quad \text{(A19)}$$

a_w is the conversion factor for mol m^{-2} s^{-1} to m s^{-1}

Table 5.A4 Parameters the Alice Holt model

	g_{max}	2.60	
$f_{PAR}= \frac{m(1.892PAR)}{g_{max} + m(1.892PAR)}$	m =7.62 x 10^{-4}		
Vapour pressure deficit (vpd) f_{vpd} = (1 – vpd/a)	a	0.04	
Soil water content (SW) $f_w = s_2\left(\frac{SW - s_1}{s_1}\right)$	s_1	32.46	s_2 4.12
Windspeed (u) fu = u/ws	ws	0.80	

Errors in estimates of R_s and R_{ns}

The overall error in each estimate of resistance will be combination of the uncertainties in the measured values and errors in the models, where they have been used. Typical estimates of errors in such results are of the order of 30 to 60%. For example analysis of the Easter Bush data showed the error in measured R_{c1_O3} was ±33% and ±25% for the modelled values. The combined error in the estimates of R_{ns} (equation A15) was therefore ±52% which is similar to the standard error (equation A20) in the block median values used to examine variations of R_{ns} with meteorological parameters (Figure 5.A2).

$$SE_x = \frac{\sigma_x}{\sqrt{(n-1)}} \qquad \text{(A20)}$$

where σ_x = the standard deviation of x, n = number of values

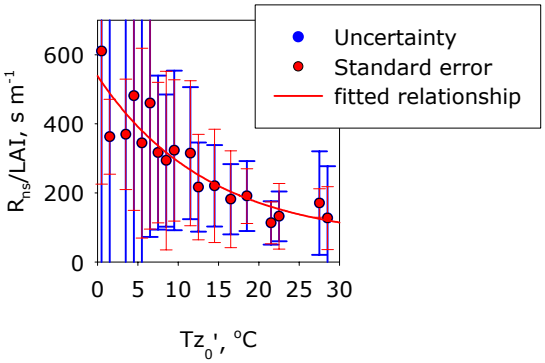


Figure 5.A2. The uncertainty in 1 °C block medians of dry R_{ns} with $T_{z0'}$ where the error bars are calculated using either the standard error of the block values or the median total uncertainty derived from the data sets

Simple Aqueous $O_3/SO_2/NH_3$ Chemistry Model

Ozone can act as an oxidising agent for SO_2 in water but this reaction is self limiting and quickly saturates when the pH is less than 6. However if ammonia is present at sufficient concentrations it can increase the pH and allow the SO_2 reaction to continue. As ozone solubility is low and the oxidation rate is very fast at high pH it can be assumed that it occurs independently of the reactions that change acidity and so a relatively simple model can be used to predict the potential ozone loss. Figure 5.A3 illustrates the process

and gives the chemical reactions (boxes A to D) that were modelled using FACSIMILE*. The model is used to predict the equilibrium pH and bisulfite concentration of the water layer and so the O_3 loss rate (box E):

$$F_{O3g\_aq} = -[L\, k_{ox} \frac{|HSO_3^-|}{\sqrt{|H^+|}} (K_{O3}|O_3|_g)]/M_{O3},\ g\ m^{-2}\ s^{-1}$$

where L = thickness of water film (m)
k_{ox} = HSO_3^- oxidation rate ($M^{-0.5}s^{-1}$)
K_{O3} = ozone solubility, 10^{-11} M ppb^{-1}
$|O_3|_g$ = gaseous ozone concentration (ppb)
M_{O3} = molecular weight of ozone, 48 g $mole^{-1}$

*The software package FACSIMILE models complex steady state and time dependent processes. It is especially suitable for solving chemical reactions with diffusion and/or advection. http://www.esm-software.com/facsimile/

Table 5.A5 Rate constants and parameter values used to model aqueous SO_2 oxidation and predict equilibrium water pH.

Parameter		Value(s)	Reference
T, water temperature		20 °C (molar volume 24 l)	
L, water film thickness		0.1 to 0.5 mm	
v_{dmax}, air/liquid maximum transfer rate		0.2 cm s^{-1}	
Gas and liquid concentrations			
$\|SO_2\|_g$	variable	0.1 to 10 ppb	
$\|NH_3\|_g$	variable	1 to 10 ppb	
$\|CO_2\|_g$	constant	340 ppm	
$\|CO_2\|_{aq}$	initial value	1.3 x 10^{-5} M	
Equilibrium constants (solubility) and reaction rates			
$K_H(SO_2)$		1.5 M atm^{-1}	Maahs 1982
$K_H(NH_3)$		92.6 M atm^{-1}	Sutton *et al* 1993
$K_H(CO_2)$		0.038 M atm^{-1}	
$K_H(O_3)$		10^{-2} M atm^{-1}	
k_{ox}		1.3 x 10^4 $M^{-0.5}s^{-1}$	Cape *et al* 1999
$K_{D1}(SO_2)$		0.014834 M	Cape 1984
$K_{D2}(SO_2)$		0.0699 x 10^{-6} M	
$K_{D1}(CO_2)$		0.417 x 10^{-6} M	
$K_{D2}(CO_2)$		4.79 x 10^{-11} M	
$K_w(H_2O)$		6.81 x 10^{-15} M	
$K_D(NH_3)$		1.71 x 10^{-5} M	

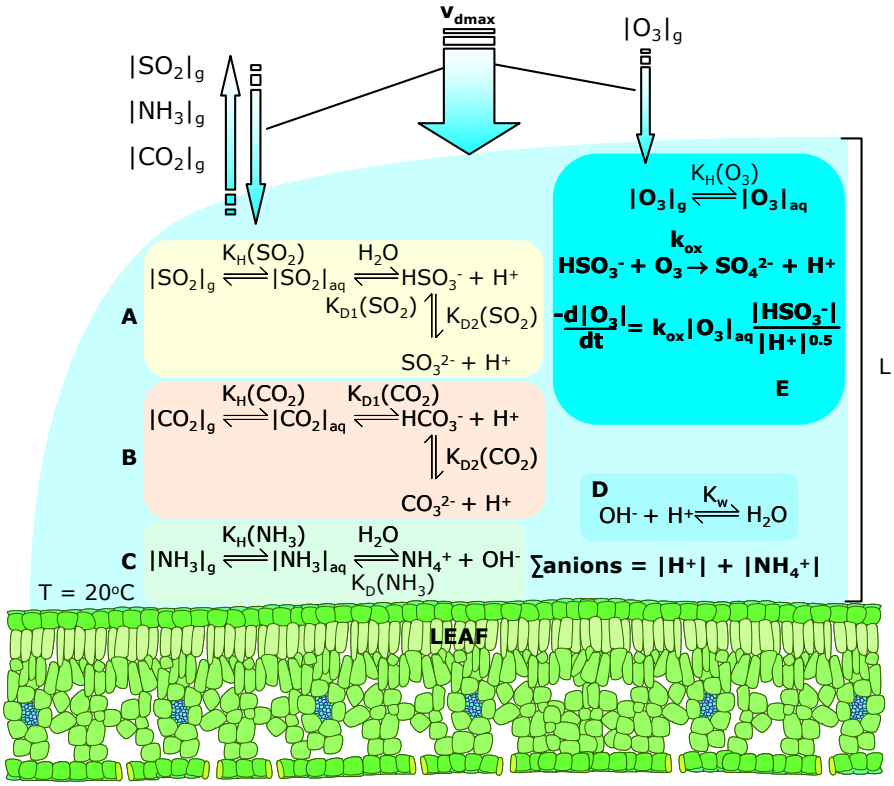


Figure 5.A3 A simple model of aqueous SO_2 oxidation used to predict the equilibrium pH of a water film. Reactions A to D are modelled until the solution reaches equilibrium and the final pH, $|HSO_3^-|$ and $|H^+|$ are used to predict the O_3 loss rate (E).

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References

Ashmore, M. R., Coyle, M. and Fowler, D., Implications of trends in background ozone concentrations for vegetation in the UK, A review for DEFRA under contract EPG 1/3/173: Development and Application of Methods for Modelling and Mapping Ozone Deposition and Flux in Europe CEH, 2002; http://www.nbu.ac.uk/pollution/docs/O3trends_UKveg.htm

Bardgett RD, Smith RS, Shiel RS, Peacock S, Simkin JM, Quirk H, Hobbs PJ (2006) Parasitic plants indirectly regulate below-ground properties in grassland ecosystems. Nature 439: 969-972

Batty, K., Ashmore, M.R., 2003. Lower threshold ozone exposures for effects on roots than shoots in wetland species, in: Karlsson et al. (Eds), Establishing ozone critical levels II, 2003 UNECE Workshop Report. IVL report B1523 IVL Swedish Environmental Research Institute, Gothenburg, Sweden. pp. 199–203.

Bender, J., Bergmann, E., Dohrmann, A., Tebbe, C.C., Weigel, H.J., 2002. Impact of ozone on plant competition and structural diversity of rhizosphere microbial communities in grassland mesocosms. Phyton-Annales Rei Botanicae 42(3), 7-12.

Bergmann, E., Bender, J., Weigel, H.J., 1995. Growth responses and foliar sensitivities of native herbaceous species to ozone exposures. Water Air and Soil Pollution 85, 1437-1442.

Bungener, P., Balls, G.R., Nussbaum, S., Geissmann, M., Grub, A., Fuhrer, J., 1999a. Leaf injury characteristics of grassland species in relation to soil moisture condition and vapour pressure deficit. New Phytologist 142, 271-282.

Bungener, P., Nussbaum, S., Grub, A., Fuhrer, J., 1999b. Growth response of grassland species to ozone in relation to soil moisture condition and plant strategy. New Phytologist 142 (2), 283-293.

Businger, J. A., Wyngaard, J. C., Izumi, Y. and Bradley, E. F., Flux-profile relationships in the atmospheric surface layer, Journal of Atmospheric Sciences, 28, 181-189, 1971

Clayton H, Knight MR, Knight H, McAinsh MR, Hetherington AM (1999) Dissection of the ozone-induced calcium signature. Plant Journal 17: 575-579

Coe, H., Gallagher, M. W., Choularton, T. W. and Dore, C., Canopy scale measurements of stomatal and cuticular o3 uptake by sitka spruce, Atmospheric Environment, 29, 1413-1423, 1995

Coyle, M., Smith, R.I., Stedman, J.R., Weston, K.J., Fowler, D., 2002. Quantifying the spatial distribution of surface ozone concentration in the UK. Atmospheric Environment 36(6), 1013-1024.

Coyle, M., Ashmore, M. R., and Fowler, D., New directions: Implications of increasing tropospheric background ozone concentrations for vegetation, Atmospheric Environment, 37, 153-154, 2003

Davison AW, Barnes JD (1986). Effects of winter stress on pollutant responses. In: How are the Effects of Air Pollutants on Agricultural Crops Influenced by the Interaction with Other Limiting Factors? Proc. COST/CEC Workshop, Roskilde p.16-32. CEC Brussels

Davison AW, Barnes JD (1988). Modifiers 10.3. Winter Stress. In: The Effects of Acid Deposition on the Terrestrial Environment in the United Kingdom. DEFRA T.E.R.G. Review, HMSO Publication Centre, London, pp. 74-77.

Davison, A.W., Barnes, J.D., 1998. Effects of ozone on wild plants. New Phytologist 139, 135-151.

De Miguel, A. and Bilbao, J., Ozone dry deposition and resistances onto green grassland in summer in central Spain, Journal of Atmospheric Chemistry, 34, 321-338, 1999

Enders, G., Deposition of Ozone to a Mature Spruce Forest - Measurements and Comparison to Models, Environmental Pollution, 75, 61-67, 1992

Finkelstein, P. L., Ellestad, T. G., Clarke, J. F., Meyers, T. P., Schwede, D. B., Hebert, E. O. and Neal, J. A., Ozone and sulfur dioxide dry deposition to forests: Observations and model evaluation, Journal of Geophysical Research-Atmospheres, 105, 15365-15377, 2000

Franzaring, J., Tonneijck, A.E.G., Kooijman, A.W.N., Dueck, T.A., 2000. Growth responses to ozone in plant species from wetlands. Environmental and Experimental Botany 44, 39-48.

Fuentes, J. D., Gillespie, T. J., Denhartog, G. and Neumann, H. H., Ozone Deposition onto a Deciduous Forest During Dry and Wet Conditions, Agricultural and Forest Meteorology, 62, 1-18, 1992

Fuhrer J., Ashmore M.R., Mills G., Hayes F. & Davison A.W. (2003). Ozone critical levels for semi-natural vegetation. In: Establishing Ozone Critical Levels II (Karlsson P.E., Selldén G. & Pleijel H., Eds.). UNECE Workshop Report B1523, IVL Swedish Environmental Research Institute, Gothenburg, Sweden.

Fumagalli, I., Mignanego, L., Mills, G., 2003. Ozone biomonitoring with clover clones: Yield loss and carryover effect under high ambient ozone levels in northern Italy. Agriculture, Ecosystems and Environment 95(1), 119-128.

Gallagher, M. W., Beswick, K. M. and Coe, H., Ozone deposition to coastal waters, Quarterly Journal of the Royal Meteorological Society, 127, 539-558, 2001

Garland, J. A. and Derwent, R. G., Destruction at the ground and the diurnal cycle of concentration of ozone and other gases, Quarterly Journal Royal Meteorological Society, 105, 169-183, 1979

Gerosa, G., Vitale, M., Finco, A., Manes, F., Denti, A. B. and Cieslik, S., Ozone uptake by an evergreen Mediterranean Forest (Quercus ilex) in Italy. Part I: Micrometeorological flux measurements and flux partitioning, Atmospheric Environment, 39, 3255-3266, 2005

Gimeno B.S., Bermejo V., Sanz J., de la Torre D. & Elvira S. (2004). Growth response to ozone of annual species from Mediterranean pastures. Environmental Pollution 132, 297-306.

Gimeno, B.S., Bermejo, V., Sanz, D., de la Torre, D., Elvira, S., 2004. Growth response to ozone of annual species from Mediterranean pastures. Environmental Pollution 132, 297-306.

Jones, B., 2001. Seed Plants, in: Rhind, P., Evans, D. (Eds), The Plant Life of Snowdonia. Gomer Press, Llandysul, Ceredigion, UK, pp. 1-36.

Lee, X., Massman, W. J. and Law, B., Handbook of Micrometeorology: A guide for surface flux measurement and analysis, Handbook of Micrometeorology, Kluwer, 2004

LRTAP Convention, 2004. Manual on methodologies and criteria for modelling and mapping critical loads & levels and air pollution effects, risks and trends. Convention on Long-range Transboundary Air Pollution. http://www.icpmapping.org

Lyons TM, Ollerenshaw JH, Barnes JD (1999) Impacts of ozone on Plantago major: apoplastic and symplastic antioxidant status. New Phytologist 141: 253-263

Lyons, T.M., Barnes, J.D., Davison, A.W., 1997. Relationships between ozone resistance and climate in European populations of *Plantago major*. New Phytologist 136, 503-510.

Mansfield TA (1998) Stomata and plant water relations: does air pollution create problems? Environ. Pollution 101, 1-11.

Matyssek R, Gunthard-Goerg M, Maurer S, Keller T (1995) Night-time exposure to ozone reduces whole plant productivity of Betula pendula. Tree Physiology 15: 159-165.

McAinsh MR, Clayton C, Mansfield TA, Hetherington AM (1996) Changes in stomatal behaviour and guard cell cytosolic free calcium in response to oxidative stress. Plant Physiology 111, 1031-1042.

McAinsh, MR, Evans NH, Montgomery LT, North KA (2002) Calcium signalling in stomatal responses to pollutants. New Phytologist 153, 441-447.

McKay, W. A., Stephens, B. A. and Dollard, G. J., Laboratory measurements of ozone deposition to sea-water and other saline solutions, Atmospheric Environment Part A-General Topics, 26, 3105-3110, 1992

Monteith, J. L. and Unsworth, M., Principles of Environmental Physics, Edward Arnold, 1990

Mortensen, L., Nilsen, J., 1992. Effects of ozone and temperature on growth of several subalpine plants species. Journal of Agricultural Science 6, 195-204.

NEGTAP, Transboundary Air Pollution: Acidification, Eutrophication and Ground-level Ozone in the UK, Report of the National Expert Group on Transboundary Air Pollution (NEGTAP) for the UK Department for Environment, Food and Rural Affairs, Scottish Executive, The National Assembly for Wales/Cynulliad Cenedlaethol Cymru and the Department of the Environment for Northern Ireland, 2001; http://edinburgh.ceh.ac.uk/negtap/

Nussbaum, S., Geissman, M., Fuhrer, J., 1995. Ozone exposure relationships for mixtures of perennial ryegrass and white clover depend on ozone exposure patterns. Atmospheric Environment 29, 989-995.

Pastori GM, Foyer CH (2002) Common components, networks and pathways of tolerance to stress. The contral role of redox and ABA-mediated controls. Plant Physiology 129, 460-468.

Paulson, C. A., The Mathematical Representation of WInd Speed and Temperature Profiles in the Unstable Atmospheric Surface Layer, Journal of Applied Meteorology, 9, 857-861, 1970

Peacock S, Smith RS, Shiel RS, Simkin JM (2004) Ecological mechanisms affecting the restoration of diversity in agriculturally-improved meadow grassland: Effect of residual soil fertility, FYM amendments and Rhinanthus minor on the biomass of grassland plant species in a mesocosm experiment. Final Report DEFRA contract BD 1439. 31 pp.

Pei ZM, Murata Y, Benning G, Thomine S, Klusner B, Allen GJ, Grill E, Schroeder JI (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature 406, 731-734.

Pleijel, H., Danielsson, H., 1997. Growth of 27 herbs and grasses in relation to ozone exposure and plant strategy. New Phytologist 135, 361-367.

Power, S.A., Ashmore, M.R., 2002. Responses of fen and fen-meadow communities to ozone. New Phytologist 156, 399-408.

Reiling, K., Davison, A.W., 1992. The response of native herbaceous species to ozone – growth and fluorescence screening. New Phytologist 120, 29-37.

Rhind, P., Evans, D., 2001. The Plant Life of Snowdonia. Gomer Press, Llandysul, Ceredigion, UK.

Rodwell, J.S., 1992 British Plant Communities Volume 3: Grassland and Montane Communities. Cambridge University Press, Cambridge, UK.

Smith RS, Shiel RS, Bardgett RD, Millward D, Corkhill P, Rolph G, Hobbs PJ, Peacock S (2003) Soil microbial community, soil fertility, plant species richness and plant community as targets in the restoration management of a meadow grassland. Journal of Applied Ecology 40: 51-64.

Soja, G., Eid, M., Gangl, H., Redl, H., 1997. Ozone sensitivity of grapevine (*Vitis vinifera* L.): Evidence for a memory effect in a perennial crop plant? Phyton-Annales Rei Botanicae 37(3), 265-270.

Soja, G., Reichenauer, T., Eid, M., Schaber, R., Gangl, H., 2003. Long-term ozone exposure and ozone uptake of grapevines in open-top chambers, in: Karlsson et al. (Eds), Establishing ozone critical levels II, 2003 UNECE Workshop Report. IVL report B1523 IVL Swedish Environmental Research Institute, Gothenburg, Sweden. pp. 104-109.

Sutton, M. A., Fowler, D. and Moncrieff, J. B., The Exchange of Atmospheric Ammonia with Vegetated Surfaces .1. Unfertilized Vegetation, Quarterly Journal of the Royal Meteorological Society, 119, 1023-1045, 1993

Timonen U., Huttunen S. & Manninen S. (2004). Ozone sensitivity of wild field layer plant species of northern Europe. A review. Plant Ecology 172, 27-39.

Tjoelker MG, Volin JC, Oleksyn J, Reich PB (1995) Interaction of ozone ollution and light effects on photosynthesisin a forest canopy experiment. Plant, Cell and Environment 18: 895-905

Volk, M. Bungener, P., Montani, M., Contat, F., Fuhrer, J. Grassland yield declined by a quarter in five years of free-air ozone fumigation. Global Change Biology, in press.

Wellburn (1994) Air pollution and climate change, Longman Scientific Publishers, UK.

Wesely, M. L., Cook, D. R. and Williams, R. M., Field Measurement of Small Ozone Fluxes to Snow, Wet Bare Soil, and Lake Water, Boundary-Layer Meteorology, 20, 459-471, 1981

Wilczak, J. M., Oncley, S. P. and Stage, S. A., Sonic Anemometer Tilt Correction Algorithms, Boundary-Layer Meteorology, 99, 127-150, 2001

Winner WE, Lefohn AS, Cotter IS, Greitner CS, Nellesen J, McEvoy LR, Olson RL, Atkinson CJ, Moore LD (1989) Plant responses to elevational gradients in ozone exposures in Virginia. Proceedings National Academy of Sciences, USA 86: 8828-8832

Zheng, Y., Lyons, T., Barnes, J., 2000. Effects of ozone on the production and utilization of assimilates in *Plantago major.* Environmental and Experimental Botany 43, 171-180.

Zheng, Y., Shimizu, H., Barnes, J.D., 2002. Limitations to CO_2 assimilation in ozone-exposed leaves of *Plantago major*. New Phytologist 155(1), 67-78.